

Malaria Pan/Pv/Pf Rapid Device

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

CAT. NO.	PRODUCT DESCRIPTION
8/608	Malaria Pan/Pv/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 utmost importance due to incidence of cerebral malarial and drug resistance associated with *P.f.* 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.f.* and *P.v.* is of utmost importance for better patient management and speedy recovery.

The BIOTEC Malaria Pan/Pv/Pf Rapid Device detection system for *P.f.* malaria is based on the detection of *P.f.* specific histidine rich protein –2 (Pf HRP-2) which is a water soluble protein released from parasitised erythrocytes of infected individuals. The detection system of *P.v.* is based on the presence of *P.v.* specific pLDH. 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 anti malarial therapy.

Principles of the Method

The BIOTEC Malaria Pan/Pv/Pf Rapid Device is a rapid immunochromatographic test. As the test sample flows through the membrane assembly of the device after addition of the clearing buffer, the coloured colloidal gold conjugates of anti-HRP-2 antibody, anti *P.v.* 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-*P.f.* HRP-2 antibody and / or monoclonal anti-*P.v.* specific pLDH antibody and / or monoclonal pan specific pLDH antibody coated on the membrane. This leads to formation of a pink / purple coloured band in the respective regions, which confirms a positive test result. Absence of a coloured band in the test region indicates a negative test result for the corresponding antigen.

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:

- Individual pouches, each containing:
 - 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.
 - Desiccant pouch.
 - 5µl sample loop.
- Clearing buffer in a dropper bottle.
- Instructions for use.

ITEMS REQUIRED BUT NOT PROVIDED

- Calibrated micropipette capable of delivering 5µl sample accurately (optional).
- Specimen collection container.
- 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

5. 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

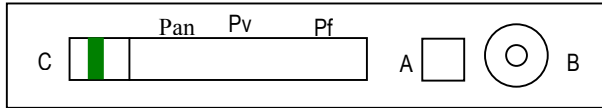
6. Alternatively, 5µl of the anti-coagulated or finger prick specimen may be delivered to the sample pad in the sample port 'A' using a micropipette.

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

7. Dispense four drops of the clearing buffer into port 'B', by holding the plastic dropper bottle vertically.
8. 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:

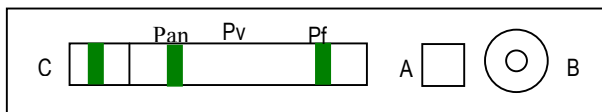
INTERPRETATION

Negative for malaria: Only one pink / pink-purple band appears at the control region 'C'.

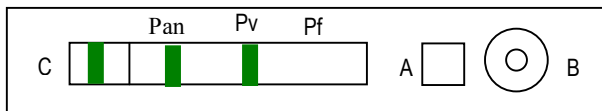


Positive for malaria:

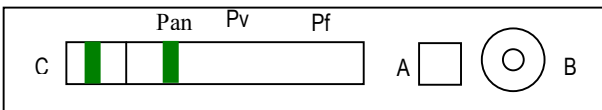
***P. falciparum* malaria:** In addition to the control band, a pink / pink-purple band appears at the 'Pf' and 'Pan' regions respectively.



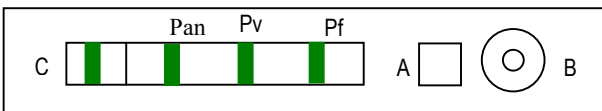
***P. vivax* malaria:** In addition to the control band, a pink / pink-purple band appears at 'Pv' and 'Pan' regions respectively.



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



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



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 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 *P.v.* 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 up to 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. 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 more. In such cases it is strongly recommended to extend the reading time by another 15 minutes so that 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/Pv/Pf Rapid Device. The results obtained are as follows:

Sample	No. samples	Malaria Pan/Pv/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%

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KEY TO SYMBOLS



Temperature limitation



Use by/Expiry date

IVD

In Vitro Diagnostic



Harmful (Xn)