diagnosticks

MALARIA *(Pv/Pf)*
Rapid Diagnostic Test
for detection of *Plasmodium vivax* and *falciparum* Malaria in human WHOLE BLOOD
Cassette

INTRODUCTION
diagnosticks - Malaria (*Pv/Pf*) is a rapid, self performing, qualitative, two site sandwich immunoassay utilizing human **whole blood** for the detection of *P. falciparum* specific histidine rich protein-2(Pf. HRP-2) and *P. vivax* specific pLDH. The test can also be used for specific detection and differentiation of *P. falciparum* and *P. vivax* malaria in areas with high rates of mixed infections.

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 considered the " Big Two" due to incidence of cerebral malaria and drug resistance associated with *P. falciparum* malaria, and high rate of infectivity and relapse associated with *P. vivax*. As the course of treatment is dependent on the species, differentiation between *P. falciparum* and *P. vivax* of utmost importance for better patient management and speedy recovery.
In ***diagnosticks*** - Malaria (*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.

PRINCIPLE
diagnosticks - Malaria (*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 monoclonal anti Pf. HRP-2 antibody and monoclonal anti Pan specific pLDH antibody complexes the HRP-2 /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 vivax specific pLDH antibody coated on the membrane leading to formation of pink-purple colored band/s which confirms a positive test result. Absence of colored band/s in the test region indicates a negative test result for the corresponding antigen. The un reacted 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 MATERIALS SUPPLIED
diagnosticks - Malaria (*Pv/Pf*) kit contains :
A. Individual pouches, each containing:
1. Cassette (Test Device) : Pre-dispensed with monoclonal anti Pf. HRP-2 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 and anti rabbit antibody at the respective regions.
2. Desiccant Pouch.
3. 5 µl Sample Loop.

B. Buffer Solution 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°C-30°C till the duration of the shelf life as indicated on the pouch/carton. DO NOT FREEZE.

NOTES
1. Read the instructions carefully before performing the test.
2. For in vitro diagnostic use only. NOT FOR MEDICINAL USE.
3. Do not use beyond expiry date.
4. Do not inter mix reagents from different lots.
5. Handle all specimens as potentially infectious.
6. Follow standard bio-safety guidelines for handling and disposal of potentially infective material and kit materials.

SPECIMEN COLLECTION AND PREPARATION
Fresh blood from finger prick / puncture should be used as a test specimen. However, fresh anti coagulated

whole blood may also 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°C- 8°C for up to 72 hours before testing. Clotted or contaminated blood samples should not be used for performing the test.

TESTING PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the ***diagnosticks***-Malaria (*Pv/Pf*) kit components to room temperature before testing.
2. Open the pouch and retrieve the device, sample loop and the desiccant. Check the color 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 in to the blood sample. Ensuring that a loop full of blood is retrieved, blot the blood so collected 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 in the sample port 'A' (Care should be taken that the blood sample has not clotted and the transfer to the sample port is immediate).

OR

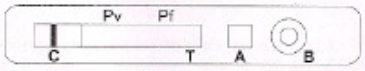
Alternatively, 5 µl of the anti coagulated or finger prick specimen may be delivered in the sample port 'A' using a micro pipette.

NOTE : Ensure that the blood from the sample loop has been completely taken up at the sample port 'A'.

5. Immediately dispense 2 drops of the buffer solution into buffer port 'B', by holding the plastic dropper bottle vertically.
6. Read the results at the end of 20 minutes as follows:

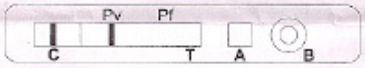
NEGATIVE :

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

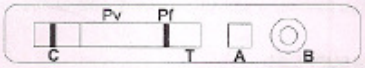


POSITIVE :

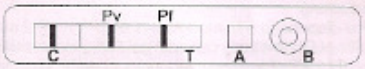
P. vivax malaria : In addition to the control band, a pink-purple band also appears under the region marked 'Pv' in the test window 'T'.



P. falciparum malaria : In addition to the control band, a pink-purple band also appears under the region marked 'Pf in the test window 'T'.



Mixed Infection : In addition to the control band, two pink-purple bands appear under the regions marked '*Pv*' & '*Pf*' in the test window 'T'.



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 results must always be correlated with clinical findings.
2. The results of the 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 used of other reagents will invalidate the test procedure.
4. Interference due to presence of heterophile antibodies in patient's sample can lead to erroneous analyte detection in immunoassay, has been reported in various studies. ***diagnosticks***-Malaria *(Pv/Pf)* uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of these interferences.
5. ***diagnosticks***-Malaria *(Pv/Pf)* is 100% sensitive to *P. falciparum* and *P. vivax* malaria. However , a negative test result does not rule out the possibility of infection with *P. ovale* and *P. malariae*.
6. In case of infection with *P. vivax* usually, the 'Pv' bands can be employed for monitoring success of 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.
7. 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.
8. In *P. falciparum* malaria infection, Pf.HRP-2 is not secreted in gametogony stage. Hence, in "Carriers",the 'Pf' band may be absent.
9. Since Pf.HRP-2 persists for up to a fortnight even after successful therapy, a positive test result does not indicate a failed therapeutic response. 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.

10. The 'Pv' band can be used for monitoring success of anti malarial therapy, in case of stand alone *P. vivax* infection. For monitoring success of anti malarial therapy in case of stand alone *P. falciparum* infection or mixed infection, employing a Pan specific pLDH based system is recommended after 5-10 days of initiation of the chemotherapeutic agent.
11. Do not interpret the test results beyond 30 minutes.

PERFORMANCE CHARACTERISTICS

In an in house study, a panel of 207 samples whose results were earlier confirmed with microscopy were tested with ***diagnosticks*** - Malaria *(Pv/Pf)* The results obtained were as follows :

Sample	Total Number of Samples Tested	***diagnosticks*** - Malaria *(Pv/Pf)*		Sensitivity (%)	Specificity (%)
		Positive	Negative		
P. falciparum Positive	22	22	0	100	-
P. vivax Positive	17	17	0	100	-
Malaria Negative	168	0	168	-	100

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