MALARIA (Pf-HRP II/Pv-pLDH) ANTIGEN CARD IMMUNOPAK

(Whole Blood)

Last update 10-2020

Ref. RDT-MAL.102N, 50 Test

INTENDED USE

Malaria Pf/Pv Ag test is a rapid, qualitative, chromatographic immunoassay for the detection of *P.falciparum* specific histidine rich protein-II (Pf, HRP-II) and *P.vivax* specific pLDH in human blood sample. The test can also be used for specific detection and differentiation of *P.falciparum* malaria and *P.vivax* malaria in areas with high rates of mixed infections.

INTRODUCTION

Malaria is a serious, sometimes fatal, parasitic disease. Four species of the Plasmodium parasite are responsible for malarial 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 causing most of the morbidity and mortality worldwide. 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 Malaria Pf/Pv Ag, the detection system for *P.falciparum* malaria is based on the detection of *P.falciparum* specific histidine rich protein-II (Pf, HRP-II), which is a water soluble protein that is released from parasitized erythrocytes of infected individuals. The detection system for *P.vivax* malaria is based on presence of *P.vivax* specific pLDH.

PRINCIPLE

Malaria Pf/Pv Ag test utilizes the principle of immune-chromatography. It contains a membrane strip, which is pre-coated with Monoclonal Anti-HRP II antibody (test line pf) specific to the Histidine Rich Protein-II of *P. falciparum* and the other with Monoclonal Anti Pan pLDH antibody (test line Pv) which is specific to the lactate dehydrogenase of Pan species . As the test sample flows through the membrane assembly of the device after addition of assay buffer (diluent), the colored colloidal gold conjugates of Monoclonal Anti-Pf, HRP II antibody and Monoclonal Anti-Pv pLDH antibody which is specific to the lactate dehydrogenase of *P. Vivax* species, complexes the lysed blood sample. This complex get immobilized on the respective test lines on the nitrocellulose membrane which leads to the formation of pink-purple line/s. A line will appear under Pf at the test region in falciparum positive samples, while a line will appear under Pv in vivax malaria positive samples. Appearance of line under Pf as well as Pv in the test region suggests a mixed infection.

To serve as a procedural control, an additional line of Goat anti-mouse IgG has been immobilized on the strip. If the test is performed correctly, this will result in the formation of pink-purple line on control line.

PRESENTATION

	50 Tests
Malaria (Pf-HRP II/Pv-pLDH) Antigen card	50 Cards
Assay Buffer	1 Bottle
Sample Dropper (5µl)	50 Droppers
Sterile Lancet	50 Nos.
Alcohol Pad	50 Nos.
Product insert	

WARNING AND PRECAUTIONS

1. Read the product insert before carrying out the test and Instructions must be followed exactly to get accurate results.
2. The device is sensitive to humidity as well as to heat. So, it's very important to take out the device from the sealed pouch just before carrying out the test.
3. Do not use the kit after the expiration date.
4. For in vitro diagnostic use only.
5. Wear protective gloves while handling samples and wash hands thoroughly after performing the test.
6. Dispose all the samples and kits properly after test in accordance with GLP.
7. Do not pipette reagent or blood sample by mouth.
8. Do not smoke, drink or eat in areas where specimen or kit reagents are being handle. Handle all specimens as potentially infectious.
9. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.

STORAGE AND STABILITY

Malaria Pf/Pv Antigen test card should be stored at 2°C-40°C. The card may be stored at room temperature but not exceeding 40°C in the original sealed pouch. The shelf life or expiry of the card is printed on Pouch as well as carton label. The shelf life of the kit is 24 months after its manufacture date. The test kit should be kept away from direct sunlight, moisture and heat.

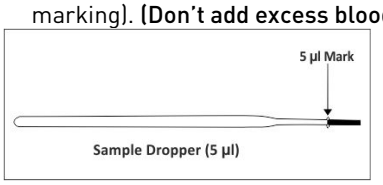
SPECIMEN COLLECTION AND STORAGE

1. The test should be performed with freshly collected human blood from the fingertip or by vein puncture using sample tube containing anticoagulant.
2. For the short term storage, please keep the specimen at 2°C-8°C, for the long term storage; please keep the sample below - 20°C.

TEST PROCEDURE

Note: - The test device should be used immediately (within 2 minutes) after removal from the pouch.

1. Clean the fingertip with the alcohol swab and let it dry completely. Prick the fingertip with a single use lancet.
2. Collect 5 µl of blood using the capillary dropper (upto indicated marking). **(Don't add excess blood).**



3. Load the 5 µl of blood into the sample well "S" of the test device.
4. Add exactly 3 drops (90 µl) of assay buffer (Diluent) into the assay buffer well "A" of the test device. **It is important to allow each drop to soak in the sample well before adding next drop of assay buffer.**
5. Immediately start the stop watch.
   Note: - After 5 minutes of adding specimen and buffer, add one more drop of assay buffer for better background clearance.
6. Read the results at the end of 20 minutes.

IMPORTANT NOTE:

Results should not be read beyond 30 minutes. Reading too late can give false results.

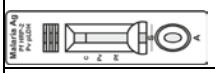
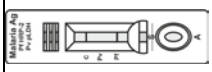
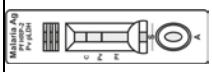
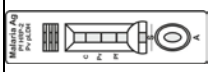
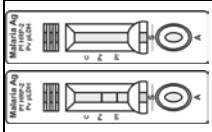
MALARIA (Pf-HRP II/Pv-pLDH) ANTIGEN CARD

IMMUNOPAK

(Whole Blood)

Last update 10-2020

INTERPRETATION OF RESULT

	**NEGATIVE for malaria:** Only one pink-purple line appears in the control area marked 'C'.
	***P. falciparum* Positive:** Control line "C" and Test line "Pf" appears in the test window. The blood sample is infected by *P. Falciparum.*
	***P. Vivax* Positive:** Control line "C" and Test line "Pv" appears in the test window. The blood sample is infected by *P. vivax*.
	***Mixed Infection*:** Along with the control line "C", the Test line "Pf" and the test line "Pv" appears in the test window. The sample is infected with *P.falciparum* and *P. vivax* infection.
	**INVALID RESULT:** If control line "C" does not appear, the test is may be invalid. In this case, Please repeat the test following the test procedure exactly.

PERFORMANCE CHARACTERISTICS

In an in-house study, a panel of 292 samples whose results were earlier confirmed with microscopy were tested with Malaria (Pf/Pv) Ag card. The results obtained are as follows:

Sample	Total No. of samples tested	Malaria (pf/pv) Ag test		Sensitivity (%)	Specificity (%)
		Positive	Negative		
P. falciparum positive	32	32	0	100	-
P. vivax positive	45	45	0	100	-
Malaria Negative	215	0	215	-	100

The sensitivity of Malaria Antigen (Pf/Pv) test is compared to microscopy absorbance with more than 200 parasites per µl and confirms to WHO (FIND) testing assessment protocol.

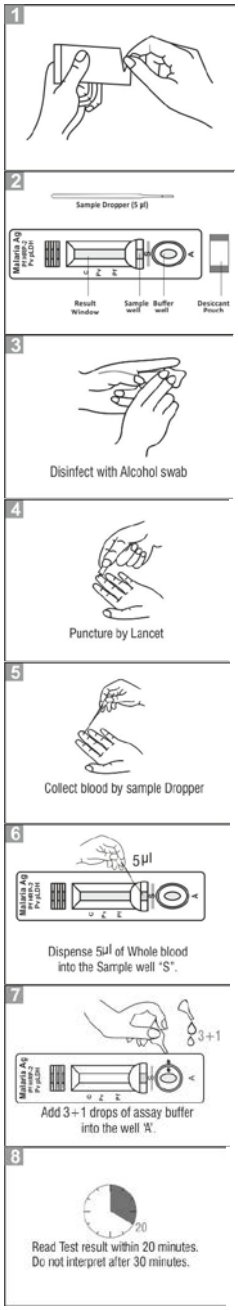
LIMITATIONS OF THE TEST

1. The test procedure, precautions and interpretation of results for this test must be followed when testing.
2. Malaria Pf/Pv Ag test is designed primary screening of malaria infection.
3. The test limited to the detection of antigen to malaria plasmodium sp. Although the test is very accurate in detecting pLDH, a low incidence of false results can occur. Other clinically available tests are required if questionable results are obtained.
4. 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).
5. Anti-coagulants such as heparin, EDTA, and citrate do not affect the test result.
6. Do not mix reagent of different lots.
7. Malaria Pf/Pv Ag test 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*.
8. In *P.falciparum* malaria infection, Pf, HRP-II is not secreted in gametogony stage. Hence in "Carriers", the 'Pf' line may be absent.

REFERENCES

1. World health Organization-Geneva (2000) new perspectives malaria diagnosis.
2. Perlmann, P and Troye-Blomberg, M.2002. Malaria parasites and Disease. Malaria immunology.
3. Malcolm, J.G., et al, 2002. Genome sequence of the human malaria parasite plasmodium falciparum, Nature 419:498-511.
4. Histidine Rich Protein II: a novel Approach to malaria Drug Sensitivity Testing Antimicrobial agents and Chemotherapy, June 2002, P.1658-1664 Vol.46, No.6.

TEST PROCEDURE



Open pouch at cut mark and remove all content, the device and desiccant pouch. Once opened, the device must be used immediately.

Component details are shown in picture.

Clean the finger to be pricked, with an alcohol swab. Allow to dry.

Take a lancet & prick the finger with the pointed end of the lancet.

Collect the blood sample up to indicated marking by sample dropper.

Load the collected blood (5 µl) in the sample well 'S' of the test device.

Hold the assay buffer vial vertically straight & add exactly 3 drops in to the well 'A'.
After 5 minutes, add one more drop of assay buffer.

Start the stop watch and read the test results at the end of 20 minutes.

Regd. Office.3/7, B.I.D.C., Gorwa, Vadodara 390 016 (INDIA)
Web: www.reckondiagnostics.com Ph: +91·265·2281631
Email: mail@reckondiagnostics.com
An ISO 13485:2016 Certified Company

RECKON DIAGNOSTICS P. LTD.
THE TEST OF CONFIDENCE - SINCE 1989