BIOGENIX
INC. PVT. LTD.

Rapid Malaria Pf / Pan Antigen Test

In-Vitro Diagnostic Use Only **Store at 2°C to 30°C Package Insert**

1. OVERVIEW

Malaria is a serious parasitic disease characterized by fever, chills, and anaemia and is caused by a parasite that is transmitted by the bite of infected Anopheles mosquitoes. There are four kinds of malaria parasites that can infect humans: Plasmodium falciparum, P. vivax, P. ovale, and P. malariae. In humans, the parasites (called sporozoites) migrate to the liver where they mature and release another form, the merozoites. At present Malaria is diagnosed microscopically using thick and thin blood films. These require expert knowledge to correctly identify the species, not always available 24hrs a day. This is where a reliable support test becomes invaluable.

2. INTENDED USE

This is a rapid, in vitro, qualitative lateral flow immunoassay for the detection of P. falciparum specific histidine rich protein-2 (Pf. HRP-2) and Pan specific aldolase from human whole blood samples. The test may also be used for the differentiation of P. falciparum and Pan infection.

3. PRINCIPLE

The Rapid Malaria Pf (HRP2)/ Pan (aldolase) Antigen Test contains a membrane strip, which is pre-coated with two test lines and one control line. One monoclonal antibody (Pf - test line 1) is specific to Pf Histidine Rich Protein 2 (HRP2) of the Plasmodium falciparum species and the other line (PAN - test line 2) consists of a monoclonal antibody specific to PAN aldolase. The control line (C) consists of Goat anti-Rabbit IgG. The conjugate pad is dispensed with HAMA blocking reagent and colloidal gold conjugated to P. falciparum specific HRP2, Pan specific aldolase
antibodies and rabbit IgG. The test is designed for the differential diagnosis between malaria specific species *Plasmodium falciparum* and *Pan*.
After addition of the blood sample and the assay buffer to the respective wells on the test containing a test strip, the whole blood gets lysed and if the sample contains detectable levels of the Pf HRP2 antigen and/or Pan aldolase antigen it reacts with the respective gold conjugated with malaria Pf specific HRP2 antibodies and/or Pan aldolase specific antibodies to form a complex. The unbound colloidal gold particles along with complex move on to the nitrocellulose membrane. This complex moves further and reacts with the respective malaria Pf specific HRP2 antibodies/ PAN specific aldolase antibodies test lines on the nitrocellulose membrane area to form a coloured bands (Test band/s). The unbound complex, unbound gold and the rabbit IgG conjugated colloidal gold particles move further to the goat-anti rabbit IgG coated control area to form a coloured band (C- Control line). The appearance of test lines and control line in respective area indicates the positive result. Appearance of only control line indicates a negative result. The control line acts as a procedural control. Control line should always appear if the test is performed as per the procedure and reagents are working properly.

4. MATERIAL PROVIDED

1. Test: Nitrocellulose Membrane assembly pre-dispensed with monoclonal anti-Pf HRP-2 antibody, monoclonal PAN aldolase antibody, Goat anti rabbit IgG, Conjugate strip containing HAMA blocking reagent and colloidal gold conjugated monoclonal anti-Pf HRP-2 antibody, Pan aldolase antibody, and rabbit IgG at the respective regions.
2. Desiccant pouch
3. Package Insert
4. Assay Buffer
5. Sample Loop

5. OPTIONAL MATERIAL REQUIRED

1. Calibrated micropipette capable of delivering 5µl sample accurately.
2. Stop watch
3. Disposable gloves

1.Please read all the information in this package insert before performing the test. Pay particular attention to the position of the Control and Test lines.
2. Do not use after the expiration date printed on the foil pouch.
3. Store in the sealed pouch in a dry place in between temperature 2°C to 30°C. Do not freeze.
4.Do not use if pouch is torn or damaged.
5. Do not open the foil pouch until you are ready to start the test.
6. Keep out of the reach of children.

7. WARNINGS

1. Do not reuse the test.
2. Follow the instruction to get accurate results.
3. Use appropriate personal protective equipment
4. Dispose of hygienically as per local regulatory requirements.
5. Do not touch the membrane.
6.Treat blood samples and used tests as potentially infectious. Avoid contact with skin.
7. For in vitro diagnostic use. Not to be taken internally.
8. Do not eat the desiccant in the package.
9. Do not mix the specimen sample or interchange the different specimen.

8. SPECIMEN COLLECTION

Fresh anti-coagulated whole blood should be used as a test sample. EDTA or Heparin or Oxalate or Tri-sodium Citrate can be used as suitable anticoagulants. The specimen should be collected in a clean glass or plastic container. If immediate testing is not possible then store the specimen at 2°C to 8°C for up to three days 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.

9. TEST PROCEDURE

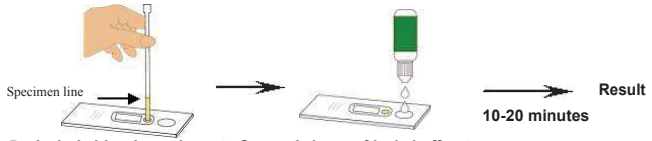
1. Bring the kit components to room temperature before testing.
2. Open the pouch and retrieve the test, sample loop and desiccant pouch. Check the color of the desiccant. Once opened, the test must be used immediately.
3.Label the test test with patient's identity
4.Tighten the vial cap of the assay buffer provided with the kit in the clockwise direction to pierce the dropper bottle nozzle.
5. 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 in the sample port 'S'. (This delivers approximately 5µl 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 'S'. (Care should be taken that the blood sample has not clotted and the transfer to the sample port is immediate).

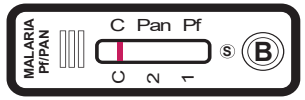
OR

Alternatively, 5µl of the anti-coagulated or finger prick specimen may be delivered in the sample port 'S' using a micro pipette.
NOTE: Ensure that the blood from the sample loop has been completely taken up at the sample port 'S'.
6. Immediately dispense three drops of assay buffer in to buffer port 'B', by holding the plastic dropper bottle vertically.
7. Read the results at the end of 20 minutes.



10. INTERPRETATION OF RESULTS

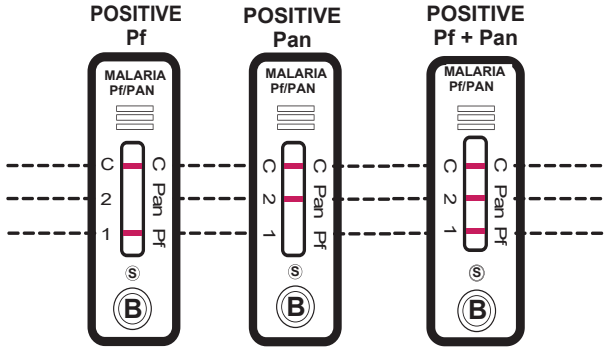
NEGATIVE for Malaria: If pink-purple coloured band appears at the control region 'C'



POSITIVE for P. falciparum: In addition to the control band, one pink-purple band appears only at region 'Pf' in the test window.

POSITIVE for Pan: In addition to the control band, one pink-purple band appears only at region 'Pan' in the test window.

POSITIVE for P. falciparum and Pan mixed infection: In addition to the control band, two pink-purple bands appear at regions 'Pf' and 'Pan in the test window.

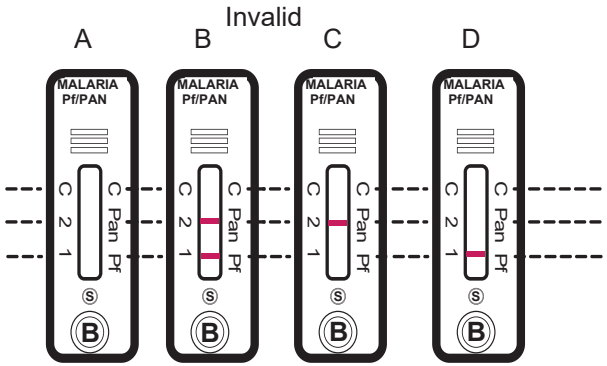


INVALID: The test should be considered invalid if
A) No line appears at 'C' , 'Pf' and 'Pan' regions.

B) No line appears at 'C' region and line appear at 'Pf' and 'Pan' region.

C) No line appears at 'C' and 'pf' region and line appear at 'Pan' region.

D) No line appears at 'C' and 'PAN' region and line appear at 'Pf' region.



11. Performance Characteristics: -

Internal Evaluation:
In an in-house study, total 200 samples were evaluated for sensitivity and specificity. We found the relative sensitivity was 100 % (i. e. 100/100) and the relative specificity was 100 % (i. e. 100/100). The results are summarized in the following table:

Sample	Total Number of samples tested	Rapid Malaria Pf (HRP 2)/ Pan (aldolase) Antigen Test		Sensitivity (%)	Specificity (%)
		Positive	Negative		
Malaria Pf Positive Whole Blood Samples	52	52	0	100	-
Malaria PanPositive Whole Blood Samples	51	51	0	100	-
Malaria Negative Whole Blood Samples	100	0	100	-	100

Cross reactivity was studied using RF positive samples and no cross reactivity was observed.

External Evaluation:
In an external study, total 200 samples were evaluated for sensitivity and specificity. Relative sensitivity was 100 % (i. e. 50/50) and the relative specificity was 100 % (i. e. 150/150). Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for the test was 100 %.

The results are summarized in the following table:

Sample	Total Number of samples tested	Rapid Malaria Pf (HRP 2)/ Pan (aldolase) Antigen Test		PPV (%)	NPV (%)
		Positives	Negative		
Malaria Pf Positive Whole Blood Samples	19	19	0	100	-
Malaria PanPositive Whole Blood Samples	31	31	0	100	-
Malaria Negative Whole Blood Samples	150	0	150	-	100

12. LIMITATIONS

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 test is limited to the detection of antigen to Malaria Plasmodium sp. Although the test is very accurate in detecting aldolase and HRP-2,
a low incidence of false results can occur. Other clinically available tests are required if questionable results are obtained. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

5. Do not interpret the test results beyond 30 minutes.

13. REFERENCES

1. David L. Vander Jagt, Lucy A. Hunsaker and John E. Heidrich: Partial Purification and Characterization of Lactate Dehydrogenase from Plasmodium falciparum. Molecular and Biochemical Parasitology, 4 (1981) 255-264

2. 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

3. Hunte-Cooke A., et. al., (1999) Comparison of a Parasite Lactate Dehydrogenase-based Immunochromatographic Antigen Detection assay (OptiMAL®) with Microscopy for the Detection of Malaria Parasites in Human Blood Samples. Am J.Trop Med 60(2). 173-176.

4. John, S. M., et. al.,(1998) Evaluation of OptiMAL , a dipstick test for the diagnosis of malaria. Ann. Trop. Med. Parasitol., 92, 621-622.

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BIOGENIX INC. PVT. LTD.

	Consult instrucon for use	LOT	Batch number
	For single use		IVD product
	Store between		Date of manufacture
	Manufacturer		Contains sufficient for 40 tests
	Keep dry		Expire date