

1. OVERVIEW

Malaria is a serious parasitic disease characterized by fever, chills, and anemia and is caused by a parasite that is transmitted by the bite of infected Anopheles mosquitoes. There are four kinds of malaria 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 malaria specific pLDH from human whole blood samples. The test may also be used for the differentiation of P. falciparum and other malarial species and for the follow up of anti-malarial therapy in whole blood samples.

3. PRINCIPLE

The Rapid Malaria Pf (HRP2)/ PAN (pLDH) Antigen test-Device contains a membrane strip, which is pre-coated with two test lines and one control line. One monoclonal antibody (Pf - test line 1) is Pf specific to 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 plasmodium lactate dehydrogenase (pLDH). 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 pLDH antibodies and rabbit IgG. The test is designed for the differential diagnosis between Plasmodium falciparum and the other Pan specific species (P. vivax, malariae and ovale).

After addition of the blood sample and the assay buffer to the respective wells on the device containing a test strip, the whole blood gets lysed and moves on to the conjugate pad containing colloidal gold particles conjugated with malaria Pf specific HRP2 antibodies, PAN specific pLDH antibodies and rabbit IgG. If the sample contains detectable levels of the Pf HRP2 antigen/ Pf pLDH antigen it reacts with the respective gold conjugated malaria Pf specific HRP2 antibodies / PAN pLDH specific antibodies to form a complex. This complex moves further and reacts with the respective malaria Pf specific HRP2 antibodies/ PAN specific pLDH antibodies test lines on the nitrocellulose membrane area to form a coloured band. The unbound complex 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 Device
2. Desiccant pouch
3. Disposable 5µl sample applicator
4. Package Insert
5. Assay Buffer

5. OPTIONAL MATERIAL REQUIRED

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

6. PRECAUTIONS/KIT STORAGE AND STABILITY

1. Please read all the information in this package insert before performing the test. Pay particular attention to the position of the C and T 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 4°C to 40°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 device.
2. Follow the instruction to get accurate results.
3. Use appropriate personal protective equipment
4. Dispose of hygienically in domestic waste.
5. Do not touch the membrane.
6. Treat blood samples and used devices as potentially infectious. Avoid contact with skin.
7. For in vitro diagnostic use. Not to be taken internally.
8. 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 device, sample loop and desiccant pouch. Check the color of the desiccant. It should be blue, if it has turned colorless or pink, discard the device and use another device. Once opened, the device must be used immediately.
3. Label the test device 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).

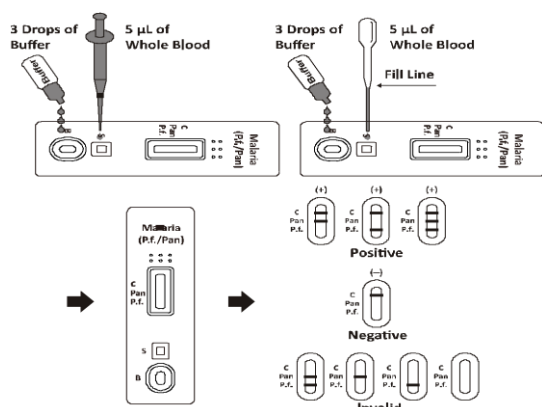
OR

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 into buffer port 'B', by holding the plastic dropper bottle vertically.
7. Read the results at the end of 20 minutes.

Test Procedure & Interpretation of results



10. INTERPRETATION OF RESULTS

(Please refer to the illustration on left)

POSITIVE:*

Two or Three distinct colored lines, including Control, appear.

P. falciparum or mixed malaria infection: one line appears in the control region, one line appears in Pan Line region and one line appears in P.f. line region.

P. falciparum infection: one line appears in the control region, and one line appears in P.f. line region.

Non-falciparum Plasmodium species infection: one line appears in the control region and one line appears in Pan Line region.

NEGATIVE: Only one colored line appears in the control region.

INVALID: Control line fails to appear.

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 (pLDH) Antigen test -Device		Sensitivity (%)	Specificity (%)
		Positive	Negative		
Malaria Pf Positive Whole Blood Samples	50	50	0	100	-
Malaria Pan Positive Whole Blood Samples	50	50	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 (pLDH) Antigen test - Device		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Positive	Negative				
Malaria Pf Positive Whole Blood Samples	19	19	0	100	-	100	-
Malaria Pan Positive Whole Blood Samples	31	31	0	100	-	100	-
Malaria Negative Whole Blood Samples	150	0	150	-	100	-	100

12. 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, the parasitological techniques of reference 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.
- 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 Uses HETEROFILIC BLOCKING REAGENT (HBR) to inhibit majority of these interferences.
- In case of mixed infection (P. falciparum with other malarial species), both, 'Pf' and 'Pan' malaria band 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.
- In P. falciparum 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 persists 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. Retesting after 2 days is advised, in such cases
- Do not interpret the test results beyond 30 minutes.

13. REFERENCES

- Howard, R.J., et al., 1986: Secretion of a Malarial Histidine-rich Protein (Pf. HRPII) from Plasmodium falciparum-infected Erythrocytes. J. Cell Biol., 103, 1269-1277
- 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.
- 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



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