In-Vitro Diagnostic Use Only **HI-Quik Rapid Malaria Pf / Pv Antigen test** Store at 4°C to 40°C

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.

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 P. vivax specific pLDH from human whole blood samples. The test may also be used for the differentiation of P. falciparum and P. vivax infection.

PRINCIPLE

The Rapid Malaria Pf (HRP2)/ Pv (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 (Pv - 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 antibodies, P. vivax specific pLDH antibodies and rabbit IgG. The test is designed for the differential diagnosis between Plasmodium falciparum and the Plasmodium vivax species.

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 HRP 2 antibodies, Pv pLDH specific antibodies and rabbit IgG. If the sample contains detectable levels of the Pf HRP 2 antigen/ Pv pLDH antigen it reacts with the respective gold conjugated malaria Pf specific HRP 2 antibodies / Pv pLDH specific antibodies to form a complex. This complex moves further and reacts with the respective malaria Pf specific HRP 2 antibodies/ PAN pLDH specific antibodies test lines on the nitrocellulose membrane area to form a coloured bands. 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.

MATERIAL PROVIDED

1. Test Device 2. Desiccant pouch 3. Disposable 5µl sample applicator 4. Package Insert 5. Assay Buffer

OPTIONAL MATERIAL REQUIRED

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

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.

WARNINGS

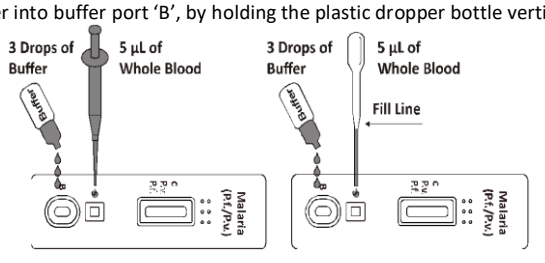
1. Do not reuse the test device.
2. Follow the instruction to get accurate results.
3. Use appropriate personal protective equipment
4. Dispose of hygienically in domestic waste.
5. Do not touch the membrane.
6. Treat blood samples and used devices as potentially infectious. Avoid contact with skin.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

SPECIMEN COLLECTION

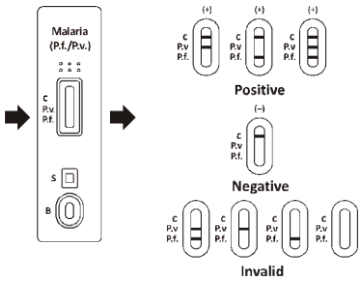
Fresh anti-coagulated whole blood should be used as a test sample. EDTA or Heparin or Oxalate or Tri-sodium Citrate can be used as suitable anticoagulants. The specimen should be collected in a clean glass or plastic container. If immediate testing is not possible then store the specimen at 2°C to 8°C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

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.**



INTERPRETATION OF RESULTS



INTERPRETATION OF RESULTS (Please refer to the illustration on left)

POSITIVE:*

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

P. vivex malaria infection: one line appears in the control region, one line appears in Pv Line region.

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

Mixed infection: one line appears in the control region and one line each appears in Pv and Pf Line region.

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

INVALID: Control line fails to appear.

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

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 pLDH 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 20 minutes.

REFERENCES

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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
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