

Rapid Malaria Pf (HRP 2) Antigen Test - Dipstick

For In-Vitro Diagnostic Use Only

Store at 4°C to 40°C

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 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) antigen from human whole blood samples.

3. PRINCIPLE

The Rapid Malaria Pf HRP-2 Antigen test-device contains a membrane strip, which is pre-coated with malaria P. falciparum Histidine rich protein 2 specific monoclonal antibody as test line (Pf) and Goat anti-rabbit IgG as control line (C).

After addition of the blood sample on to the conjugate pad of the test and dipping the test in the assay buffer, the whole blood gets lysed and if the sample contains detectable levels of the Pf (HRP-2) antigen it reacts with the gold conjugated with malaria Pf anti HRP-2 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 malaria Pf (HRP-2) specific antibodies coated at test line on the nitrocellulose membrane area to form a colored band (Test band). 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 colored 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. CONTENTS OF KIT

1. Test Dipstick: Nitrocellulose Membrane assembly pre-dispensed with monoclonal Pf. anti- HRP-2 antibody, Goat anti-rabbit IgG, Conjugate strip containing colloidal gold conjugated anti-Pf. HRP-2 antibody and rabbit IgG at the respective regions.
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
4. Transparent round bottom glass /polystyrene tubes (75 mm X 12 mm)

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 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 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.
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 remove the test and desiccant pouch. Check the color of the desiccant. It should be blue, if it has turned colorless or pink, discard the test and use another test. Once opened, the test dipstick must be used immediately.
3. Label the test dipstick 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 on the pink-purple area of the test dipstick. (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 on the pink-purple area of the test dipstick. (Care should be taken that the blood sample has not clotted and the transfer on the pink-purple area of the test dipstick is immediate).

OR

Alternatively, 5µl of the anti-coagulated or finger prick blood specimen may be delivered on the conjugate pad area of the test dipstick using a micro pipette.

- NOTE:** Ensure that the blood from the sample loop has been completely delivered on to the conjugate pad area of the test dipstick.
6. By holding the dropper bottle vertically dispense six drops (Approx. 180 µl) of assay buffer in a round bottom glass or polystyrene test tube (12 X 75) mm.
 7. Place the test dipstick in the test tube with the arrows pointing downwards.
 8. Read the results at the end of 20 minutes. Do not read the test results beyond 30 minutes.

10. INTERPRETATION OF RESULTS

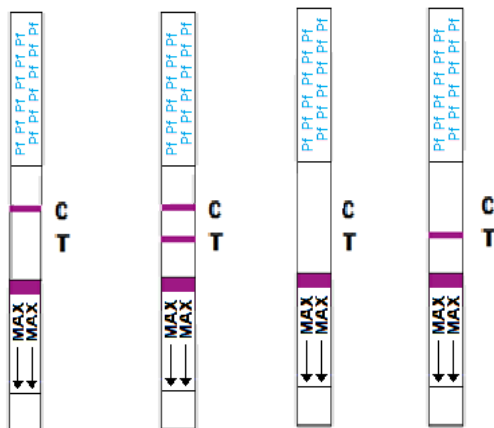
NEGATIVE for malaria: If a colored band appears at the control region 'C'

POSITIVE for P. falciparum malaria: In addition to the control band, a colored band appears at test region 'T'

INVALID: The test should be considered invalid if,

A) No line appears at control region 'C' and test region 'T'

B) No line appears at control region 'C' and line appear only at test region 'T'



NEGATIVE POSITIVE INVALID INVALID

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. 52/52) and the relative specificity was 100 %

The results are summarized in the following table:

Sample	Total Number of samples tested	Rapid Malaria Pf HRP 2 Antigen Test - Dipstick		Sensitivity (%)	Specificity (%)
		Positive	Negative		
Malaria Pf Positive Whole Blood Samples	52	52	0	100	-
Malaria Negative Whole Blood Samples	148	0	148	-	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. 19/19) and the relative specificity was 100 % (i. e. 181/181). 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 Antigen Test - Dipstick		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Positive	Negative				
Malaria Pf Positive Whole Blood Samples	19	19	0	100	-	100	-
Malaria Negative Whole Blood Samples	181	0	181	-	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. Rapid Malaria Pf HRP2 Antigen test-Device uses HETEROPHILIC BLOCKING REAGENT to inhibit majority of these interferences.

5. In *P. falciparum* malaria infection, HRP-2 is not secreted in the gametogamy stage. Hence, in "Carriers", the HRP-2 band may be absent.

6. HRP-2 levels, post treatment persists up to 15 days, *P. falciparum* malaria cases.

13. REFERENCES

- David R. and et. al. A Longitudinal Study of Type-Specific Antibody Responses to Plasmodium falciparum Merozoite Surface Protein -1 in an Area of Unstable Malaria in Sudan. Journal of Immunology, 161:347-359 (1998).
- Howard, R.J. et al, 1986.: Secretion of Malarial Histidine-rich protein (Pf HRP 2) 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.
- Parra, M.E., et al, 1991: Identification of Plasmodium falciparum Histidine-Rich Protein 2 in the Plasma of Humans with Malaria. J. Clin. Microbiol., 29. 1629-1934

	In Vitro Diagnostic Use
	Manufacturer
	Manufacturing Date
	Expiry Date
	Lot Number
	Store at 4°C to 40°C
	Single Use
	Number of tests in the pack
	Do not use if pouch or kit damaged
	This side Up
	Read product insert before use



MANUFACTURED BY

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