

MALARIA RAPID

AccuTest™ Malaria

Rapid immunodiagnostic test for the detection of circulating Malaria antigens in whole blood

Detection of
Plasmodium falciparum,
Plasmodium vivax, *Plasmodium*
orale and *Plasmodium malariae*

For In Vitro Diagnostic Use Only



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NAME AND INTENDED USE

The Malaria Rapid Test is a rapid, qualitative test for the detection of *Plasmodium falciparum* and/or *Plasmodium vivax*, *Plasmodium orale* and *Plasmodium malariae* antigen in whole blood. This test is for In-Vitro Diagnostic use

INTRODUCTION

Malaria is one of the world's most prevalent parasitic diseases and ranks third in the world among major infectious diseases in terms of mortality. The protozoal parasites that cause malaria are from the *Plasmodium* genus. Four species of *Plasmodium* protozoa cause malaria: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. Transmitted principally by the Anopheles mosquito, malaria infections may also occur from contacting infected blood, such as from blood transfusions.

P. falciparum accounts for the majority of infections and is the most lethal. *P. vivax*, *P. malariae* and *P. ovale* cause a less severe form of malaria with intermittent fever which is usually neither debilitating nor fatal. Classic symptoms of malaria include fever, headaches, chills, vomiting, shivering and convulsions. In some rare forms of falciparum malaria, chills and fever may be absent and the patient may present with delirium or coma. Remission periods can last from a few weeks to several months. Severe anemia is often attributed to the cause of death from a malaria infection.

Malaria is a curable disease with a host of drugs that can be used in both its treatment and prevention. Two of the best known and most commonly used are chloroquine and quinine. The early detection of *P falciparum* malaria is of great importance due to rising levels of drug resistance now being associated with this disease.

TEST PRINCIPLE

The Malaria Rapid test is a rapid, *in-vitro* immunodiagnostic test for the detection of circulating Malaria antigens in whole blood. The test uses antibodies that are specific for the histidine-rich protein-2 antigen (HRP-2) of Malaria *P.f.* and Plasmodium aldolase for the detection of all malaria Plasmodium species.

Whole blood (5 µl) is applied to the sample pad where the red blood cells are lysed with a specially formulated solution. The label pad that is next to the sample pad on the strip is impregnated with blue latex that has an anti-HRP-2 antibody coupled to it, along with a second blue latex that has an anti-aldolase antibody coupled to it. The label pad is also impregnated with purple latex that is coupled to a control antibody. An additional anti-HRP-2 antibody is immobilized on the test strip at

the "*P.f.* test" line region. Another anti-aldolase antibody is immobilized on the test strip at the "All test" line region. Finally, a control material is immobilized on the strip at the "Control" line region. When a positive sample is applied to the sample pad, malaria antigen in the sample contacts the latex-labeled antibody and binds to it. A washing reagent is then added to a test vial, and the strip is placed in the vial. As the liquid flows along the length of the strip, any antigen-latex complexes also migrate with the liquid. These complexes are captured by their respective antibodies at the *P.f.* All and Control line regions. If a sample contains *P.f.* antigen, a blue line will form in the *P.f.* test region and may or may not form in the All test regions, depending on the titer of the antigen present. If the sample contains *P.v.*, *P.o.* or *P.m.* antigen, a test line will form in the All test region. If no malaria antigen is present, a blue line will not form in either *P.f.* or All test region. A purple control line will always appear in the Control region if the test has been performed properly.

MATERIALS SUPPLIED

- 25 test devices in individual foil pouches
- 25 sample collection capillaries
- 1 bottle of Lysing / Wash reagent
- 1 Product insert

MATERIALS REQUIRED BUT NOT SUPPLIED:

- Lancets
- Disinfecting, sterile wipe
- Glass, borosilicate or plastic tubes, 12 x 75 mm preferred
- Timer capable of timing from 0 to 60 minutes.

STORAGE AND SHELF LIFE OF REAGENTS:

Store the kit between 2°C and 30°C. Do not store the kit in direct sunlight. Be sure to un-pouch only the number of test device that will be immediately used. The test kit may be used until its expiration date, which can be found on the package label.

PRECAUTIONS:

1. Specimens should be handled as being potentially infectious. The Centers for Disease Control (CDC) and the National Institutes of Health (NIH) recommend that all potentially infectious agents be handled at a Biosafety Level.
2. Biological decontamination procedures should be followed for all equipment, containers, surfaces, etc. that come in contact with potentially infectious specimens. All disposables that come in contact with these samples should be disposed of as infectious waste.

3. For best results, strict adherence to these instructions is required. Be careful not to touch the tip of the wash bottle to the sample tube when adding buffer to the tube. This will greatly minimize the likelihood of contaminating the wash reagent.
4. The wash solution contains a low concentration of sodium azide as a preservative (less than 0.1 %). Sodium azide is toxic. Do not drink this buffer. Sodium azide may also react with lead and copper in plumbing to form explosive compounds. If you dispose of this buffer down a drain, flush the drain with excess amounts of water to minimize the accumulation of potentially explosive metal-azide compounds.
5. Do not use the test strips or reagents beyond the stated expiration date marked on the package label.
6. Store the test kits and reagents according to temperature and humidity conditions stated on the package label.
7. All test strips, buffers and specimens must be at room temperature (15-30°C) before running the assay
8. Do not re-use the test strips or buffer.

SPECIMEN COLLECTION:

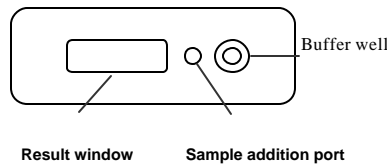
1. Handle all specimens as capable of transmitting infectious diseases. Dispose of all materials that come in contact with the specimen as infectious waste.
2. Specimens should be collected aseptically by fingerstick or venipuncture according to standard methods such as those specified by the National Committee for Clinical Laboratory Standards (NCCLS). The use of grossly lipemic or turbid samples should be avoided.
3. Whole blood samples should be used immediately, if possible. NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H1SA. 1990).
4. Use the collection capillary provided to deliver a 5µl sample or collect venous blood into EDTA tubes. To obtain capillary blood, puncture a finger, heel or other appropriate site. First cleanse the area with a disinfecting sterile wipe. Use a lancet to puncture the skin. Allow a blood droplet to form. Touch the collection capillary to the blood droplet and transfer to the test strip immediately. To collect venous blood, use the standard venipuncture procedure and collect blood into an EDTA tube. If the test cannot be performed immediately, the blood

may be stored for up to three days at 2°C to 8°C.

TEST PROCEDURE:

- Just prior to use, remove a device from the foil pouch. Lay the device flat on the work surface.
- Using a sterile lancet and clean sample capillary, collect blood in capillary tube as specified above or use 5 µl of EDTA venous blood. Ensure that the blood sample warms to room temperature prior to use.
- Transfer the blood sample from the capillary tube to the test strip by holding the capillary vertically and gently touching the full end against the pad within the sample addition port until all of the blood has been transferred. Discard the capillary properly. If using a micro-pipetter, slowly apply 5 µl of blood to the sample pad.
- Add five drops of the Lysing / Wash reagent to the buffer well.
- Using a timer, allow the reaction to proceed for 15 minutes. Do not pick up the device during this time.
- When the 15-minute period is over, read the results. If there is still a reddish background, lay the device flat on the work surface and wait an additional 15 minutes. The results may be read from 15 to 30 minutes. Do not read results after 60 minutes.

Negative results must be confirmed at 30 minutes.



IMPORTANT NOTICE:

Occasionally, residual malaria antigen may be detected for several days following elimination of the parasite by anti-malarial treatment. The diagnosis of Malaria should be made using the results of this test together with the other clinical and laboratory findings.

INTERPRETATION OF THE RESULTS:

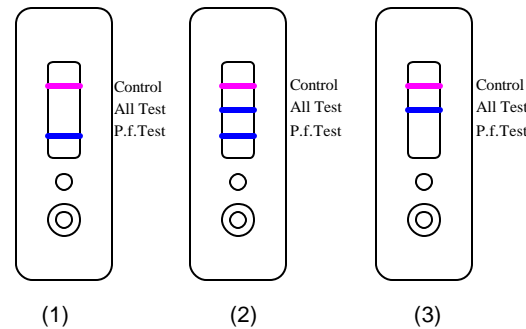
This test device contains two test result lines: A test line that solely detects Malaria *P.f.* and a test line that detects all four Malaria Plasmodium species: *P.f.*, *P.v.*, *P.o.* and *P.m.* A positive result is indicated when any visible line forms in the result window next to the *P.f.* or *All* zone together with a line in the C zone. The test is positive even if the line in the *P.f.* or *All* zone appears lighter or

darker than the line in the C zone.

- The test is not valid if the control line does not appear, regardless of the presence of line in the *P.f.* or *All* test line regions. Repeat the test with a new strip.
- Positive results may appear as early as 5 minutes. Negative results must be confirmed at 30 minutes.
- The background of the strip should be white, not red, prior to confirming a negative result.
- Results should not be read after 60 minutes.

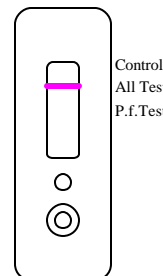
Positive Test Result - Detection of *Plasmodium falciparum* and / or *Plasmodium vivax*, *Plasmodium orale* or *Plasmodium malariae*

- A visible blue test line on the strip located in the *P.f.* zone below the control line indicates a positive test result for *Plasmodium falciparum*. The purple control line must also be present.
- A visible blue test line on the strip located in the *P.f.* zone and the All test zone indicates a positive test result for *P. falciparum*, *P. vivax*, *P. orale*, *P. malariae*. The control line must also be present.
- A visible blue test line on the device only located in the *All test* zone indicates a positive test result for *Plasmodium vivax*, *Plasmodium orale* or *Plasmodium malariae*. The test cannot distinguish between these three malaria subtypes. The control line must also be present.



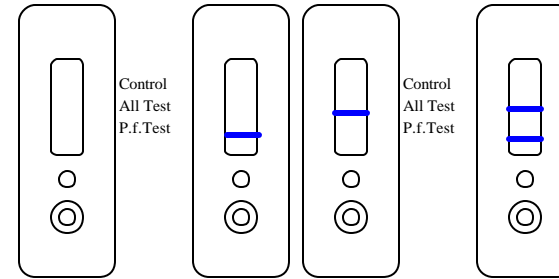
Negative Test Result

The test is negative if only the control line appears.



Invalid Test Result

The test is invalid if a purple line does not appear in the control zone. If this occurs, the test should be repeated using a new device.



EXPECTED VALUES

Histidine-rich protein 2 (HRP-2) is secreted by *Plasmodium falciparum* at the blood stages of a falciparum malaria infection. Its presence usually indicates a falciparum malaria infection. Occasionally, residual *P.f.* HRP-2 antigen may be detected for several days following elimination of the parasite by anti-malarial treatment. The diagnosis of Malaria *P.f.* should be made using the results of this test together with the other clinical and laboratory findings.

Plasmodium Aldolase is secreted by all four *Plasmodium* species. Its presence usually indicates a malaria infection. Occasionally, residual aldolase may be detected for several days following elimination of the parasite by anti-malarial treatment. The diagnosis of Malaria should be made using the results of this test together with the other clinical and laboratory findings.

QUALITY CONTROL

- For the assay to be considered valid, the control line must appear. If it does not appear, the test results are not valid and the test must be repeated.
- In addition to your laboratory's standard quality control procedures, the NCCLS recommends that a positive and negative external control to be tested at least once within each 25 tests kit and by each operator performing testing within a kit. This will verify that the reagents and test strips are working properly and the operator is able to correctly perform the test procedure. Please refer to this NCCLS publication C24-A for recommendations on appropriate Quality Control practices.

LIMITATIONS OF THE TEST:

- HRP-2 tests may give positive malaria results for up to 2 weeks following

chemotherapy and parasite clearance as confirmed by microscopy.

- As with all diagnostic tests, the result must be correlated with clinical findings. If the test result is negative and malaria infection suspicion still exists, additional follow-up testing using other clinical methods is recommended.
- A negative result at any time does not preclude the possibility of an early malaria infection.
- Strict adherence to the test procedure is required. Do not re-use negative strips. Do not adulterate the buffer reagents.
- This test cannot be used to monitor therapy or to estimate the titer of the infection.
- A final diagnosis should be based on these test results in conjunction with other clinical and laboratory findings.

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