MALARIA RAPID

AccuTest™ Malaria

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

Detection of Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae

For In Vitro Diagnostic Use Only

NAME AND INTENDED USE

The Malaria Rapid Test is a rapid, qualitative test for the detection of Plasmodium falciparum and/or Plasmodium vivax, Plasmodium ovale 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 is the third in order of magnitude among major infectious diseases in terms of mortality. The protozoan parasites that cause malaria are from the Plasmodium genus. Four species of Plasmodium protozoa cause malaria: Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae. Transmitted principally by the Anopheline mosquito, malaria infections may also occur from contact with infected blood, such as from blood transfusions.

Pl. 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 pyrimethamine.

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. falciparum and Plasmodium aldolase for the detection of malaria Plasmodium species.

Whole blood ($\mu$l) 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-aldolase 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 strip, any antigen-latex complexes also migrate with the liquid. These complexes are captured by their respective antibodies at the P.f. and All 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 region, depending on the titer of the antigen present. If the sample contains P.v., P.o. or P.m. antigen, a 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 wipes
- Glass, boric 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 test kit outside the stated expiration date marked on the package label.

PRECAUTIONS:

- 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).
- Use the collection capillary provided to deliver a $\mu$l 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 sample 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.

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

TEST PROCEDURE:
1. Just prior to use, remove a device from the foil pouch. Lay the device flat on the work surface.
2. 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.
3. 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.
4. Add five drops of the Lysing / Wash reagent for 15 minutes. Do not pick up the device during this time.
5. 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.

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

Positive Test Result - Detection of Plasmodium falciparum and / or Plasmodium vivax
Plasmodium ovo or Plasmodium malariae
1. 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.
2. A visible blue test line on the strip located in the P.v. 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.
3. A visible blue test line on the device only located in the All test zone indicates a positive test result for Plasmodium vivax, Plasmodium ovo or Plasmodium malariae. The test cannot distinguish between these three malaria subtypes. The control line must also be present.

EXPECTED VALUES
Histoiline-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 aldolase 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 Alkalase 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
1. 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.
2. 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:
1. HRP-2 tests may give positive malaria results for up to 2 weeks following chemotherapy and parasite clearance as confirmed by microscopy.
2. 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.
3. A negative result at any time does not preclude the possibility of an early malaria infection.
4. Strict adherence to the test procedure is required. Do not re-use negative strips. Do not adulterate the buffer reagents.
5. This test cannot be used to monitor therapy or to estimate the titer of the infection.
6. A final diagnosis should be based on these test results in conjunction with other clinical and laboratory findings.

REFERENCES:
5. World Health Organization Fact Sheet (1998), Malaria, No.94