Reliable Pro-detect Biomedicals Pvt. Ltd.

In vitro Diagnostic

INTENDED USE

The ***Reliable one step*** Malaria Pf/Pv Ab Rapid Test device is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of antibodies including IgG, IgM and IgA to *Plasmodium falciparum* (*Pf*) and *vivax*, *ovale*, and *malariea* (*Pv.o.m*) in human serum or plasma. This device is intended to be used as a screening test and as an aid in the diagnosis of infection with *Plasmodium* Any reactive specimen with the ***Reliable one step*** Malaria Pf/Pv Ab Rapid Test device must be confirmed with alternative testing method(s) and clinical findings.

SUMMARY AND EXPLANATION OF THE TEST

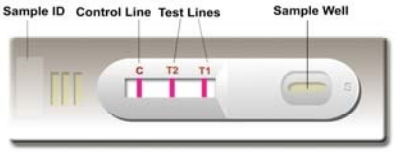
Malaria is a mosquito-borne, hemolytic, febrile illness that infects over 200 million people and kills more than 1 million people per year. It is caused by four species of *Plasmodium*: *P. falciparum, P. vivax, P. ovale, and P. malariae*. These plasmodia all infect and destroy human erythrocytes, producing chills, fever, anemia, and splenomegaly. *P. falciparum* causes more sever disease than the other plasmodial species and accounts for most malaria deaths. *P. falciparum and P. vivax* are the most common pathogens, however, there is considerable geographic variation in species distribution[1].

Traditionally, malaria is diagnosed by the demonstration of the organisms on Giemsa stained smears of peripheral blood, and the different species of plasmodium are distinguished by their appearance in infected erythrocytes[1]. The technique is capable of accurate and reliable diagnosis, but only when performed by skilled microscopists using defined protocols[2], which presents major obstacles for the remote and poor areas of the world.

The ***Reliable one step*** Malaria Pf/Pv Ab Rapid Test device is developed for solving these above obstacles. It detects the antibodies generated in serum or plasma in response to the infection of plasmodium. Utilizing the Pf. specific antigen (HRP-II) and pan-malaria antigen (aldolase), the test enables simultaneous detection and differentiation of the infection of *P. falciparum and or P. vivax, ovale, and malaria* [3-5], by untrained or minimally skilled personnel, without laboratory equipment.

TEST PRINCIPLE

The ***Reliable one step*** Malaria Pf/Pv Ab Rapid Test device is a lateral flow chromatographic immunoassay. The test device consists of: 1) a burgundy colored conjugate pad containing recombinant HRP-II andaldolase conjugated with colloid gold (*Pf* conjugates and *Pan-malaria* conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing two test bands (T1 and T2 bands) and a control band (C band). The T1 band is pre-coated with recombinant antibodies to *Pf* HRP-II antigen for the detection of only, T2 band is pre-coated with aldolase for the detection of antibodies to *Pan-malaria* protozoa, and the C band is pre-coated with goat anti rabbit IgG.



When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. Antibodies including IgG, IgM and IgA to *P. falciparum* infection, if present in the specimen will bind to the *Pf* conjugates. The immunocomplex is then captured on the membrane by the pre-coated P.f. antigen, forming a burgundy colored T1 band, indicating a *Pf* positive test result.

Alternatively, antibodies including IgG, IgM and IgA against aldolase, generated following the infection by the either form of malaria protozoa if present in the specimen will bind to the *Pan-malaria* conjugates. The immunocomplex is then captured by the pre-coated aldolase antigen on the membrane, forming a burgundy colored T2 band, indicating a plasmodium antibody positive result.

Absence of any T bands (T1 and T2) suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti-rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on any of the T bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Each kit contains 50 test devices, each sealed in a foil pouch with three items inside:
   a. One cassette device.
   b. One plastic dropper.
   c. One desiccant.
2. Sample diluent (2 bottle, 5 mL)
3. One package insert (instruction for use).

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15°C-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolized blood specimen for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as biohazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 15 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test device unopened at 2°C-30°C. The positive and negative controls should be kept at 2°C-8°C**.** If stored at 2°C-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Plasma

1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by veinpuncture.
2. Separate the plasma by centrifugation.
3. Carefully withdraw the plasma into new pre-labeled tube.

Serum

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2°C-8°C if not tested immediately.

Store specimens at 2°C-8°C up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

Step 2: When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

Step 3: Be sure to label the device with specimen's ID number.

Step 4: Fill the plastic dropper with the specimen.

Holding the dropper vertically, dispense 4 drop (about 30-45 µL) of specimen into the sample well making sure that there are no air bubbles.

Then add 1 drop (about 35-50µL) of Sample Diluent immediately.

4 drop of specimen **1 drop of sample diluent** **15 minutes**

Step 5: Set up the timer.

Step 6: Results can be read in 15 minutes. Positive results can be visible in as short as 1 minute.

Don't read result after 15 minutes. To avoid confusion, discard the test device after interpreting the result

QUALITY CONTROL

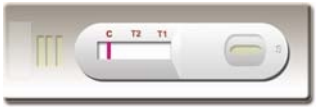
Using individual *Reliable one step* Malaria Pf/Pv Ab Rapid Test device as described in the Assay Procedure above, run 1 positive control and 1 Negative Control (both provided upon request) under the following circumstances to monitor test performance:

1. A new operator uses the kit, prior to performing testing of specimens.
2. A new test kit is used.
3. A new shipment of kits is used.
4. The temperature used during storage of the kit falls outside of 2°C-30°C.
5. The temperature of the test area falls outside of 15°C-30°C.

Expected results are as follows:

Negative Control

Only the C band shows color development, the two T bands (T1 and T2) show no color development.



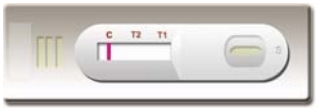
Positive Control

The C band and two T bands (T1 and T2) show color development.

The appearance of any burgundy color in the T bands, regardless of intensity, must be considered as presence of the band.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT**: If only the C band is present, the absence of any burgundy color in the both T bands (T1 and T2) indicates that no anti-plasmodium antibodies are detected. The result is negative.



2. **POSITIVE RESULT**:

2.1 In addition to the presence of C band, if only T1 band or both T1 and T2 band are developed, the test indicates for the presence of antibodies to *Pf* in the specimen. The result is *Pf* positive.

2.2 In addition to the presence of C band, if only T2 band is developed, the test indicates for the presence of antibodies to Pf, *P. vivax, oval, and or malariae* in the specimen. The result is positive.

Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. **INVALID:** If no C band is developed, the assay is invalid regardless of any burgundy color in the T bands as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. Clinical Performance For Pf Ab Test

A total of 324 patient samples from susceptible subjects were tested by the *Reliable one step* Malaria Pf/Pv Ab Rapid Test device and by a commercial Pf EIA kit. Comparison for all subjects is showed in the following table.

	Reliable one step Malaria Pf/Pv (Ab) Rapid Test		
EIA	**Positive**	**Negative**	**Total**
Positive	22	2	24
Negative	3	297	300
Total	25	299	324

Relative Sensitivity: 91.2%, Relative Specificity: 99.0%, Overall Agreement: 98.5%

2. Clinical Performance For Pv Ab Test

A total of 25 *Pv* positive samples diagnosed by microscopic examination and 300 non-malaria samples were tested by the *Reliable one step* Malaria Pf/Pv Ab Rapid Test device. Comparison for all subjects is showed in the following table.

	Reliable one step Malaria Pf/Pv (Ab) Rapid Test		
Clinic	**Positive**	**Negative**	**Total**
Positive	23	2	25
Negative	3	297	300
Total	26	299	325

Relative Sensitivity: 92 % , Relative Specificity: 99.0%, Overall Agreement: 98.5%

LIMITATIONS OF TEST

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of antibodies to plasmodium protozoa in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *Reliable one step* Malaria Pf/Pv Ab Rapid Test device is limited to the qualitative detection of antibodies to plasmodium protozoa in human serum or plasma. The intensity of the test band does not have linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable anti-plasmodium protozoa antibodies. However, a negative test result does not preclude the possibility of exposure to or infection with plasmodium protozoa.
4. A negative result can occur if the quantity of the anti- plasmodium protozoa antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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