Reliable Pro-detect Biomedicals Pvt. Ltd.

In vitro Diagnostic

INTENDED USE

The ***Reliable one step*** MALARIA Pf/Pv Ag Rapid Test device is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of *Plasmodium falciparum* (*Pf*) and *vivax (Pv)* antigen in human blood specimen. 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 Ag Rapid Test device must be confirmed with alternative testing method(s) and clinical findings.

SUMMARY AND EXPLANATION OF THE TEST

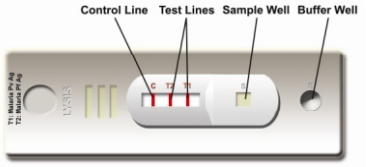
Malaria is a mosquito-borne, hemolytic, febrile illness that infects over 200 million people and kills more than 1 million people per year. It is caused by four species of *Plasmodium*: *P. falciparum, P. vivax, P. ovale, and P. malariae*. These plasmodia all infect and destroy human erythrocytes, producing 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 thick 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 Ag Rapid Test device is developed for solving these obstacles. It utilizes antibodies specific to *P. falciparum* Histidine Rich Protein-II (pHRP-II) and to *P. vivax* Lactate Dehydrogenase (Pv-LDH) to simultaneously detect and differentiate infection with *P. falciparum* and *P. vivax*[-5]. The test can be performed by untrained or minimally skilled personnel, without laboratory equipment

TEST PRINCIPLE

The ***Reliable*** one step MALARIA Pf/Pv Ag Rapid Test device is a lateral flow chromatographic immunoassay. The strip test components consist of: 1) a burgundy colored conjugate pad containing mouse anti-Pv-LDH antibody conjugated with colloid gold (Pv-LDH-gold conjugates) and mouse anti-pHRP-II antibody conjugated with colloid gold (pHRP-II-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 another mouse anti-Pv-LDH specific antibody for the detection of *Pv* infection, the T2 band is pre-coated with polyclonal anti-pHRP-II antibodies for the detection of Pf infection, and the C band is coated with go at anti-mouse IgG.



During the assay, an adequate volume of the blood specimen is dispensed into the sample well (S) of the test cassette, a lysis buffer is added to the buffer well (B). The buffer contains a detergent that lyses the red blood cells and releases various antigens, which migrate by capillary action across the strip held in the cassette. Pv-LDH if presents in the specimen will bind to the Pv-LDH-gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-Pv-LDH antibody, forming a burgundy colored T1 band, indicating a *Pv* positive test result.

Alternatively, pHRP-II if presents in the specimen will bind to the pHRP-II-gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-pHRP-II antibodies, forming a burgundy colored T2 band, indicating a *Pf* positive test result.

Absence of any T bands (T1 and T2) suggests a negative result. The test contains an internal control © band) which should exhibit a burgundy colored band of the immunocomplex of goat anti- mouse IgG / mouse IgG (anti-Pv-LDH and anti-pHRP-II)-gold conjugates 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 desiccant.
2. 50 x 5 µL mini plastic droppers
3. Blood Lysis buffer (2 bottle, 10 mL)
4. 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℃-30℃) 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. Hemolized blood may be used for the testing, but do not take precipitants.
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 30 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 30 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, preferably at 2℃-30℃. Do not expose the kit over 40℃. Do not freeze the kit. The positive and negative controls should be kept at 2℃-8℃. If stored at 2℃-8℃, 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 if it is stored at 2℃-30℃.

SPECIMEN COLLECTION AND HANDLING

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

Collect whole blood in a clean container containing anti-coagulant (EDTA, citrate or heparin) by venipuncture. Blood can be obtained by finger tip puncture as well.

Whole blood specimen should be stored in refrigeration (2℃-8℃) if not tested immediately for up to 3 days. The specimen should be frozen at -20℃ for longer storage. Avoid repeat freeze and thaw.

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. Blood will be hemolyzed after thawing.

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

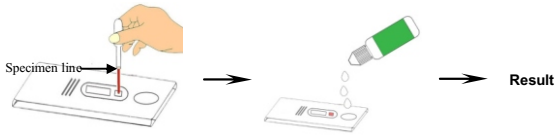
Step 3: Be sure to label the device with specimen's ID number.

Step 4: Fill in the mini plastic dropper with the blood specimen not to exceed the specimen line as shown in the following image. The volume of the specimen is around 5 µL (App. 1 drop)

Note: Practice a few times prior to testing if you are not familiar with the mini dropper. For better precision, transfer specimen by pipette capable to deliver 5µL of volume.

Holding the dropper vertically, dispense all of the specimen into the center of the sample well making sure that there are no air bubbles.

Then add 3 drops (about 100-150 µL) of Lysis Buffer immediately.



5 µL of blood specimen (App. 1 Drop) **3 drops of lysis buffer** **20-30 minutes**

Step 5: Set up timer.

Step 6: Results can be read in 20 to 30 minutes. It may take more than 20 minutes to have the background become clearer.

Don't read results after 30 minutes. To avoid confusion, discard the test device after interpreting the result

QUALITY CONTROL

Using individual ***Reliable one step*** MALARIA Pf/Pv Ag 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 fall outside of 2℃-30℃.
5. The temperature of the test area falls outside of 15℃-30℃.

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 theC band is present, the absence of any burgundy colorin the both T bands (T1 and T2) indicates that no anti-plasmodium antigens are detected. The result is negative.

2. **POSITIVE RESULT**:

2.1 In addition to the presence of C band, if only T1 band is developed, the test indicates for the presence of Pv-LDH antigen. The result is Pv positive.

2.2 In addition to the presence of C band, if only T2 band is developed, the test indicates for the presence of pHRP-II antigen. The result is Pf positive.

2.3 In addition to the presence of C band, both T1 and T2 bands are developed, the test indicates for the presence of both Pv-LDH and pHRPII antigens. The result is both Pv and Pf 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 Ag Test

A total of 224 samples from susceptible subjects were tested by the *Reliable one step* MALARIA Pf/Pv Ag Rapid Test and by thick blood smear test. Comparison forall subjects is showed in the following table.

	Reliable one step MALARIA Pf/Pv (Ag) Rapid Test		
Smear test	**Positive**	**Negative**	**Total**
Positive	24	0	24
Negative	3	197	200
Total	27	197	224

Relative Sensitivity: 100%, Relative Specificity: 98.5%, Overall Agreement: 98.7%

2. Clinical Performance For Pv Ag Test

A total of 224 samples from susceptible subjects were tested by the *Reliable one step* MALARIA Pf/Pv Ag Rapid Test and by thick blood smear test. Comparison forall subjects is showed in the following table.

	Reliable one step MALARIA Pf/Pv (Ag) Rapid Test		
Smear test	**Positive**	**Negative**	**Total**
Positive	9	0	9
Negative	3	212	215
Total	12	212	224

Relative Sensitivity: 100% , Relative Specificity: 98.6%, Overall Agreement: 98.7%

3. Cross-Reactivity

Pv and Pf cross reaction:

The negative blood specimen was spiked with 1.3 mg/mL of the recombinant Pv-LDH, Pf-LDH and pHRP-II antigen, and tested with the *Reliable* one step MALARIA Pf/Pv Ag Rapid Test, respectively. The result showed that the Pv detection system did not cross-react to the Pf detection, vise versa

Recombinant antigen Concentration	Pf- Reactivity (T2 band)	Pv- Reactivity (T1 band)
1.3 mg/mL pHRP-II	Positive	Negative
1.3 mg/mL Pv-LDH	Negative	Positive
1.3 mg/mL Pf-LDH	Negative	Negative

Cross reaction with common microbe antigens

The negative blood specimen was spiked with antigens from common microbes and then tested according to the standard procedure. The results showed that the *Reliable* one step MALARIA Pf/Pv Ag Rapid Test had no cress-reaction with the following antigens at the concentration tested.

Antigen (Ag)	Concentration spiked	Pf Reactivity (T 2 band)	Pv Reactivity (T1 band)
HIV gp 120/41 Ag	0.2 mg/mL	Negative	Negative
HIV gp 36 Ag	0.5 mg/mL	Negative	Negative
HCV Core Ag	0.75 mg/mL	Negative	Negative
HCV NS3 Ag	1 mg/mL	Negative	Negative
HBsAg	1 mg/mL	Negative	Negative
T. Palladium Ag	0.3 mg/mL	Negative	Negative
M. Tuberculosis Ag	0.2 mg/mL	Negative	Negative
Leptospria Ag	2 mg/mL	Negative	Negative
Dengue virus envelope Ag	0.1 mg/mL	Negative	Negative
Chikunguya virus Ag	2 mg/mL	Negative	Negative

4. Interference:

The common substances (such as pain and fever medication, blood components), or other infectious disease stage may affect the performance of the *Reliable one step* MALARIA Pf/Pv Ag Rapid Test device.This was studied by spiking of these substances or disease stage serum to the three levels of the pHRP-II and pLDH standard control. The results are presented on the following table and demonstrate that the substances or diseases studieddid not affect the performance of the Reliable one step MALARIA Pf /Pv Ag Rapid Test device .

Note: +::Weak positive; ++: Medium positive; +++: Strongpositive

Potential interfering substances		Pf Reactivity (T 2 band)			Pv Reactivity (T 1 band)		
		Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Control		+	++	+++	+	++	+++
Albumin	2000 mg/dL	+	++	+++	+	++	+++
Caffeine	1000 mg/dL	+	++	+++	+	++	+++
Glucose	2000 mg/dL	+	++	+++	+	++	+++
Hemoglobin	100 mg/dL	+	++	+++	+	++	+++
Human IgG	000 mg/dL	+	++	+++	+	++	+++
EDTA	0.5 mM	+	++	+++	+	++	++
Heparin	2000 mg/dL	+	++	+++	+	++	+++
Penicillin G	1000 mg/dL	+	++	+++	+	++	+++
Ketones	2000 mg/dL	+	++	+++	+	++	+++
Dengue positive serum		+	++	+++	+	++	+++
HBsAg positive serum		+	++	+++	+	++	+++
HCV positive serum		+	++	+++	+	++	+++
HIV positive serum		+	++	+++	+	++	+++
Syphilis positive serum		+	++	+++	+	++	+++
TB positive serum		+	++	+++	+	++	+++

LIMITATIONS OF TEST

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of plasmodium protozoa antigen in whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *Reliable one step* MALARIA Pf/Pv Ag Rapid Test device is limited to the qualitative detection of plasmodiumprotozoa antigen in whole blood. The intensity of the test band does not have linear correlation with the antigen titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable malaria plasmodium antigen. 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 plasmodium protozoaantigen present in the specimen is below the detection limits of the assay , or the antigen 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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