BIOGENIX
INC. PVT. LTD.

Malaria Pf/Pv Ag Rapid Test (Whole Blood)

INTENDED USE

The *Biogenix* Malaria Pf/Pv Ag Rapid Test 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 *Biogenix* Malaria Pf/Pv Ag Rapid Test 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 severe 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 .

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 . The technique is capable of accurate and reliable diagnosis, but only when performed by skilled microscopists using defined protocols , which presents major obstacles for the remote and poor areas of the world.

The *Biogenix* Malaria Pf/Pv Ag Rapid Test 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* .

TEST PRINCIPLE

The *Biogenix* Malaria Pf/Pv Ag Rapid Test 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 colloidal gold (Pv-LDH-gold conjugates) and mouse anti-pHRP-II antibody conjugated with colloidal gold (pHRP-II-gold conjugates), 2) a nitrocellulose membrane strip containing two test bands (1 & 2 bands) and a control band (C band). The band 2 is pre-coated with another mouse anti-Pv-LDH specific antibody for the detection of *Pv* infection, the band 1 is pre-coated with polyclonal anti-pHRP-II antibodies for the detection of Pf infection, and the C band is coated with goat anti-mouse IgG.
During the assay, an adequate volume of the blood specimen is dispensed into the sample well (S) of the test cassette, and 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 present 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 band 2, indicating a *Pv* positive test result.

Alternatively, pHRP-II if present 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 band 1, indicating a *Pf* positive test result.

Absence of any test bands 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-mouse IgG / mouse IgG (anti-Pv-LDH and anti-pHRP-II)-gold conjugates regardless of the color development on any of the test bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
   c. Sample loop
2. Blood Lysis buffer (1 bottle, 5mL)
3. One package insert (instruction for use)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or Timer
2. Alcohol swabs
3. Lancets or safety lancets
4. Gloves

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. 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 well 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°C-30°C. Do not expose the kit over 30°C. Do not freeze the kit. The positive and negative controls should be kept at 2°C-8°C or the temperature recommended. 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 if it is stored at 2°C-30°C.

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°C-8°C) if not tested immediately for up to 3 days. The specimen should be frozen at -20°C for longer storage. Avoid repeat freeze and thaw cycles.

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.

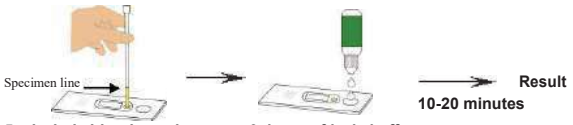
Step 3: Be sure to label the device with specimen's ID number.

Step 4: Fill the blood transfer device (sample loop, mini plastic dropper or capillary tube) with the blood specimen not to exceed the specimen line as shown in the following images. The volume of the specimen is around 5 µL.

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

Holding the blood transfer device (sample loop, mini plastic dropper or capillary tube) vertically, dispense the entire specimen into the center of the sample well making sure that there are no air bubbles.

Then add 3 drops (about 50-100 µL) of Lysis Buffer immediately.



5 µl whole blood specimen to S well **3 drops of lysis buffer to B well**

Step 5: Set up timer.

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

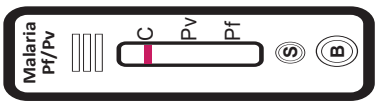
***Don't read results after 30 minutes. To avoid confusion, discard the test device after interpreting the result*.**

QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C band. The C line develops after adding specimen extract. Otherwise, review the whole procedure and repeat test with a new device.
2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. New operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kit is used.
   c. A new shipment of kits is used.
   d. The temperature used during storage of the kit falls outside of 2-30°C.
   e. The temperature of the test area falls outside of 15 -30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT**: If only the C band is present, the absence of any burgundy color in both test bands (Pv and Pf) indicates that no plasmodium antigens are detected. The result is negative.



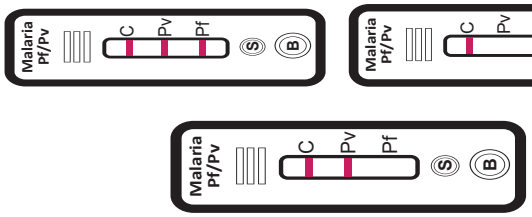
2. **POSITIVE RESULT**:

2.1 In addition to the presence of the C band, if only the band 2 is developed, the test indicates the presence of Pv-LDH antigen. The result is Pv positive.

2.2 In addition to the presence of the C band, if only the band 1 is developed, the test indicates the presence of pHRP-II antigen. The result is Pf positive.

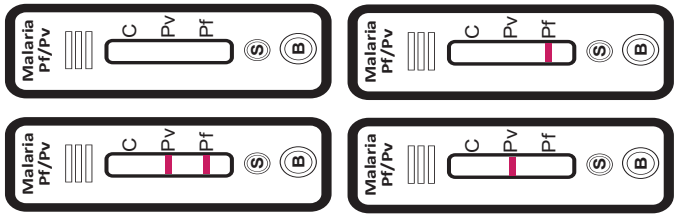
2.3 In addition to the presence of the C band, both the bands 1 & 2 are developed, the test indicates the presence of both Pv-LDH and pHRP-II antigens. The result is both Pv and Pf positive.

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 test bands as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. Clinical Performance

A total of 200 blood samples were collected from a malaria endemic area, Matiranga of Khagrachhari district of Bangladesh, and tested by the *Biogenix* Malaria Pf/Pv Ag Rapid Test and by thick blood smear test. Comparison for all subjects is shown in the following table.

	Pf		Pv	
	Positive	Negative	Positive	Negative
Smear test	94	106	7	193
***Biogenix* Malaria Pf/Pv Ag Rapid Test**	85	115	9	191

Pf detection: Sensitivity: 91.6%, Specificity: 97.9%;
Pv detection: Sensitivity: 100%, Specificity: 99%; Kappa value: 0.89

2. Cross-Reactivity

Pv and Pf cross reaction:

The negative blood specimen was spiked with recombinant Pv-LDH, Pf-LDH and pHRP-II antigen, and tested with the *Biogenix* Malaria Pf/Pv Ag Rapid Test, respectively. The result showed that the Pv detection system did not cross-react to the Pf Ag and vice versa.

Antigen Concentration	Pf- Reactivity	Pv - Reactivity
1.0 mg/mL pHRP-II	Positive	Negative
1.0 mg/mL Pv-LDH	Negative	Positive
1.0 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 *Biogenix* Malaria Pf/Pv Ag Rapid Test had no cross-reaction with the following antigens at the concentration tested.

Antigen (Ag)	Concentration Spiked	Pf Reactivity	Pv Reactivity
HIV-1 P24 Ag	1.0 mg/mL	Negative	Negative
HBsAg	1.0 mg/mL	Negative	Negative
Dengue virus NS1 Ag (I, II,III,IV)	1.0 mg/mL	Negative	Negative
Chikungunya virus Ag	1.0 mg/mL	Negative	Negative

Cross reactivity with specimens from other infectious diseases:

Specimen	Sample size	Pf Reactivity	Pv Reactivity
Dengue serum	10	Negative	Negative
HBsAg serum	10	Negative	Negative
HAV serum	10	Negative	Negative
HCV serum	10	Negative	Negative
HIV serum	10	Negative	Negative
Syphilis serum	10	Negative	Negative
TB serum	10	Negative	Negative
H. pylori serum	10	Negative	Negative
ANA serum	8	Negative	Negative
HAMA	19	Negative	Negative
RF (≤2,500 IU/ml)	10	Negative	Negative

3. Interference:

Common substances (such as pain and fever medication, blood components) may affect the performance of the *Biogenix* Malaria Pf/Pv Ag Rapid Test. This was studied by spiking of these substances to the three levels of the pHRP-II and Pv-LDH standard control. The results are presented in the following table and demonstrate that the substances studied did not affect the performance of the *Biogenix* Pf/Pv Ag Rapid Test.

Note: -:Negative; +: Weak positive; +++: Strong positive

Potential interfering substances spiked	Pf Reactivity			Pv Reactivity		
	Negative	Weak Positive	Strong Positive	Negative	Weak Positive	Strong Positive
Control	-	+	+++	-	+	+++
Bilirubin 20 mg/dL	-	+	+++	-	+	+++
Creatinine 442 µmol/L	-	+	+++	-	+	+++
Glucose 55 mmol/L	-	+	+++	-	+	+++
Albumin 60 g/L	-	+	+++	-	+	+++
Salicylic acid 4.34 mmol/L	-	+	+++	-	+	+++
Heparin 3,000 U/L	-	+	+++	-	+	+++
EDTA 3.4 µmol/L	-	+	+++	-	+	+++
Human IgG 150 mg/dL	-	+	+++	-	+	+++

WASTE MANAGEMENT OR DISPOSABLE:

The contents of RDTs can be divided into :

Infectious waste:

- sharps (lancets, needles, scalpel blades)
- blood collection devices (tubes, straws, and loops); gloves; swabs; and cotton
- used cassettes.

Non-infectious waste (Recyclable):

- packaging materials, desiccant, buffer, and unused or unusable RDTs.

You must collect and dispose each type of waste in separate containers as per your waste management policies.

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assy Result sections 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 *Biogenix* Malaria Pf/Pv Ag Rapid Test is limited to the qualitative detection of plasmodium protozoa 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 protozoa antigen present in the specimen is below the detection limits of the assay or the antigens that are detected are not present during the stage of disease in which a sample is collected.
5. A recent study showed that due to their genetic diversity some Pf isolates collected in the Peruvian Amazon lack the HRP2 gene . Therefore, a negative result in the band 1 may not rule out infection of Pf in this area.
6. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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