



LAB - STIX
DIAGNOSTICS (Pty) Ltd.

In vitro Diagnostic
Catalog Number: R0113C

INTENDED USE

The Pf / Pan Malaria Ag Rapid Test is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of *Plasmodium falciparum* (Pf) antigen and *P. vivax*, *P. ovale*, or *P. malariae* 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 Pf / Pan Malaria Ag Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

SUMMARY AND EXPLANATION OF THE TEST

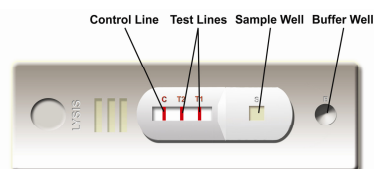
Malaria is a mosquito-borne, hemolytic, febrile illness that infects over 200 million people and kills more than 1 million people per year. It is caused by four species of *Plasmodium*: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. These plasmodia all infect and destroy human erythrocytes, 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 distribution¹.

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 erythrocytes¹. The technique is capable of accurate and reliable diagnosis, but only when performed by skilled microscopists using defined protocols², which presents major obstacles for the remote and poor areas of the world.

The Pf / Pan Malaria Ag Rapid Test is developed for solving these obstacles. The test utilizes a pair of monoclonal and polyclonal antibodies to *P. falciparum* specific protein, Histidine Repeat Protein II (pHRP-II), and a pair of monoclonal antibodies to plasmodium Lactate Dehydrogenase (pLDH), a protein produced by the four species of the plasmodium, thus enables simultaneous detection and differentiation of the infection with *P. falciparum* and or any of the other three plasmodia³⁻⁶. It can be performed by untrained or minimally skilled personnel, without laboratory equipment.

TEST PRINCIPLE

The Pf / Pan Malaria Rapid Test is a lateral flow chromatographic immunoassay. The test strip components consist of: 1) a burgundy colored conjugate pad containing mouse anti-pHRP-II antibody conjugated with colloidal gold (pHRP II-gold conjugates) and mouse anti-pLDH antibody conjugated with colloidal gold (pLDH-gold conjugates), 2) a nitrocellulose membrane strip containing two test bands (T1 and T2 bands) and a control band (C band). T1 band is pre-coated with monoclonal anti-pLDH antibody by which the infection with any of the four species of plasmodia can be detected, 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 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, a lysis buffer is added to the buffer well (B). The buffer contains a detergent that lyses the red blood cells and releases various plasmodium antigens, which migrate by capillary action across the strip held in the cassette. 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.

pLDH if presents in the specimen will bind to the pLDH gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-pLDH antibody, forming a burgundy colored T1 band, indicating a plasmodium positive test result. In the absence of T2 band, a positive test result for any of the other three plasmodia can be recommended.

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-mouse IgG / mouse IgG (pHRP-II and pLDH-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

- Each kit contains 30 test devices, each sealed in a foil pouch with four items inside:
 - One cassette device.
 - One 5 μ L mini plastic dropper.
 - One sealed plastic dropper containing blood Lysis buffer
 - One desiccant.
- One package insert (instruction for use).

MATERIALS REQUIRED AND AVAILABLE FOR PURCHASE

- Positive Control (1 vial, red cap, 1 mL)
- Negative Control (1 vial, green cap, 1 mL)

MATERIALS REQUIRED BUT NOT PROVIDED

- Clock/watch or Timer
- Lancing device or finger tip puncture device for taking blood specimen
- Clean scissors for cutting tip of the dropper containing lysis buffer

WARNINGS AND PRECAUTIONS

For in Vitro Diagnostic Use

- This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
- Do not open the sealed pouch, unless ready to conduct the assay.
- Do not use expired devices.
- Bring all reagents to room temperature (15°C-30°C) before use.
- Do not use the components in any other type of test kit as a substitute for the components in this kit.
- Hemolyzed blood may be used for the testing, but do not take precipitants.
- Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
- Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Dispose of all specimens and materials used to perform the test as biohazardous waste.
- Handle the Negative and Positive Control in the same manner as patient specimens.
- 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.
- 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 40°C. Do not freeze the kit. 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 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

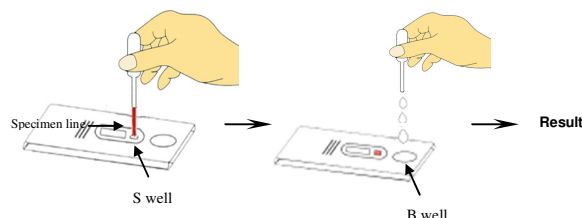
ASSAY PROCEDURE

- 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.
- When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.
- Be sure to label the device with specimen's ID number.
- Fill in the mini plastic dropper with the blood specimen not to exceed the specimen line as showed in the following image. The volume of the specimen is around 5 μ L.

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 5 μ L of volume.

Holding the dropper vertically, dispense all of the specimen into the center of the sample well (S well) making sure that there are no air bubbles. Then immediately cut the tip of the dropper containing sample diluent with scissors and add 6 drops of lysis buffer into the buffer well (B well).

Note: be sure to check for air bubbles close to the dispensing tip of the dropper. Remove any air bubbles at the tip by squeezing a few drops of liquid out. Then immediately squeeze 6 drops of lysis buffer into the buffer well (B well).



Add 5 μ L of blood specimen into the S well

Add 6 drops of lysis buffer into the B well

20-30 minutes

Step 5: Set up clock/watch or 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

INTERPRETATION OF ASSAY RESULT

- 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 plasmodium antigens are detected. The result is negative.



- 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 pLDH antigen. The result is either Pv, Pm, or Po 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 pHRP-II and pLDH. The result is Pf positive (Subject **Limitations of Test -3**).



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

- 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

- Clinical Performance with Pf positive specimen**

A total of 224 samples from susceptible subjects were tested by the Malaria Pf/Pan Malaria Ag Rapid Test and by thick blood smear test. Comparison for all subjects is showed in the following table.

Smear test	Malaria Pf/Pan Malaria Ag Rapid Test		Total
	Positive	Negative	
Positive	24	0	24
Negative	3	197	200
Total	27	197	224

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

- Clinical Performance with Pv positive specimen**

A total of 224 samples from susceptible subjects were tested by the Malaria Pf/pan Malaria Ag Rapid Test and by thick blood smear test. Comparison for all subjects is showed in the following table.

Smear test	Malaria Pf / Pan malaria Ag Rapid Test		Total
	Positive	Negative	
Positive	9	0	9
Negative	3	212	215
Total	12	212	224

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

- External Evaluation**

The Pf /Pan Malaria Ag rapid test was evaluated by the Research Institute for Tropical Medicine, a WHO affiliation in Philippines. The result is showed in the following table:

Quality control dilutions		Pf /Pan malaria Ag Rapid Test		
Sample ID	(parasites/μl)	Device tested	Positive result	% positive
P5F2 (Pf)	200	2	2	100%
	2000	1	1	100%
P5F4 (Pf)	200	2	2	100%
	2000	1	1	100%
P5F5 (Pf)	200	2	2	100%
	2000	1	1	100%
P5F8 (Pf)	200	2	2	100%
	2000	1	1	100%
P5V2 (Pv)	200	2	2	100%
	2000	1	1	100%
P5V5 (Pv)	200	2	2	100%
	2000	1	1	100%
P5V7(Pv)	200	2	2	100%
	2000	1	1	100%
P31(Pv)	200	2	2	100%
	2000	1	1	100%
Negative control	0	Device tested	Negative result	% negative
		1	1	100

LIMITATIONS OF TEST

- 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.
- The Pf / Pan Malaria 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.
- In the case of co-infection with Pf and any of the other three plasmodia, both T1 and T2 band will be developed. Thus, interpret the result cautiously when both T1 and T2 bands are visible.**
- A negative result for an individual subject indicates absence of detectable plasmodium protozoa antigen. However, a negative test result does not preclude the possibility of exposure to or infection with plasmodium protozoa.
- 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 antigen that are detected are not present during the stage of disease in which a sample is collected.
- If the symptom persists, while the result from Pf / Pan Malaria Ag Rapid Test is negative, it is recommended to re-test the specimen with an alternative test device.
- Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
- The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

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Distributed by Labstix Diagnostics Pty Ltd
 P O Box 904520, Faerie Glen, 0043
 Tel: +27 13 947 8049 / Fax: +27 86 669 7760
info@labstix.co.za / www.labstix.co.za