

INTENDED USE

The TRUSTline Malaria Pf/Pan Ag Rapid Test is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of *Plasmodium falciparum* (Pf) antigen and *P. vivax* (Pv), *P. ovale* (Po), or *P. malariae* (Pm) antigen in human blood specimen. This device is intended to be used by professionals as a screening test and provides a preliminary test result to aid in the diagnosis of infection with plasmodium.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

SUMMARY AND EXPLANATION OF THE TEST

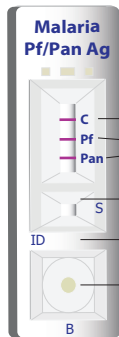
Malaria is a mosquito-borne, hemolytic, febrile illness that infects over 200 million people and kills more than 1 million people per year. It is caused by four species of *Plasmodium*: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. All *Plasmodium* spp. infect and destroy human erythrocytes and lead to chills, fever episodes, anemia, and splenomegaly. *P. falciparum* causes more severe disease than the other *Plasmodium* 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 performed only by well-trained microscopists using defined protocols², which presents major obstacles for the remote and poor areas of the world.

The TRUSTline Malaria Pf/Pan Ag Rapid Test is developed for solving these obstacles. The test utilizes a pair of antibodies to detect *P. falciparum* Histidine-rich protein II (pHRP-II), and a pair of antibodies to detect the plasmodium Lactate Dehydrogenase (pLDH) for detection of *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*, thus enabling simultaneous detection and differentiation of an infection with *P. falciparum* and/or any of the other three plasmodium species^{3,6}. It can be performed within 30 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE

The TRUSTline Malaria Pf/Pan Ag Rapid Test is a lateral flow chromatographic immunoassay. The strip in the test cassette consists of: 1) a burgundy colored conjugate pad containing monoclonal anti-pHRP-II antibody conjugated with colloidal gold (pHRP-II-gold conjugates), monoclonal anti-pLDH antibody conjugated with colloidal gold (pLDH-gold conjugates) and a control antibody conjugated with colloidal gold and 2) a nitrocellulose membrane strip containing two test lines (Pan and Pf lines) and a control line (C line). The Pan line is pre-coated with anti-pLDH antibody for the detection of infection with any of the four species of plasmodium, the Pf line is pre-coated with anti-pHRP-II antibodies for the detection of Pf infection, and the C line is coated with a control line antibody.



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 plasmodium antigens which migrate by capillary action across the strip held in the cassette.

The 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 Pf line, indicating a Pf positive test result.

The pLDH, if present 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 Pan line. In the presence of a Pf line, a Pan line indicates a positive result for Pf or a positive result for Pf and any of the other three *Plasmodium* species (Pv, Pm, Po). In the absence of a Pf line, a Pan line indicates a positive result for Pv, Po or Pm or a combination of any of these three *Plasmodium* species.

Absence of any test lines (Pan and Pf) suggests a negative result. The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of the control antibodies regardless of the color development on any of the test lines. If the C line does not develop, the test result is invalid, and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

- Individually sealed foil pouches containing:
 - One cassette device
 - One desiccant
- 5 µL blood transfer devices
- Blood lysis buffer bottle (5 mL/bottle)
- One package insert (instruction for use)

MATERIALS REQUIRED BUT NOT PROVIDED

- | | |
|--|----------------------|
| 1. Clock or Timer | 3. Alcohol swab |
| 2. Lancing device for whole blood test | 4. Disposable gloves |

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 (15-30 °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 use 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 bio-hazardous waste.
- Handle the Negative and Positive Control in the same manner as patient specimens.
- The testing results should be read 30 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside 30 minutes should be considered invalid and must be repeated.
- Do not perform the test in a room with strong air flow, i.e. 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 1-30 °C. If stored at 2-8 °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 to temperatures above 30 °C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them with standard bio-safety procedures.

Drops of whole blood can be obtained by either finger tip puncture or venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®).

Whole blood specimen should be stored at 2-8 °C for up to 3 days if not tested immediately. The specimen should be frozen at -20 °C for longer storage. Avoid multiple freeze-thaw cycles.

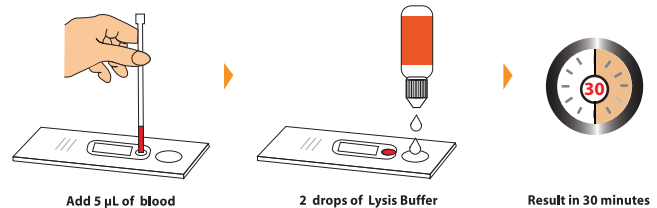
ASSAY PROCEDURE

- Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once the specimen is thawed, mix well prior to performing the assay. 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 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 5 µL volume.

Holding the blood transfer device vertically, dispense the entire specimen into the center of the sample well (S well) making sure that there are no air bubbles.

Then immediately add 2 drops of Blood Lysis Buffer (50-100 µL) into center of the buffer well (B well) with the bottle positioned vertically.



- Step 5: Set up timer.
- Step 6: Results can be read at 30 minutes. It may take more than 20 minutes to have the background become clearer. However, results must be confirmed at the end of the 30 minutes only. **Any results interpreted outside 30 minutes should be considered invalid and must be repeated. Discard used device after interpreting the result following local laws governing the disposal of device.**

QUALITY CONTROL

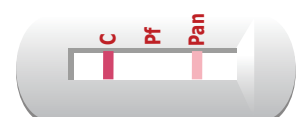
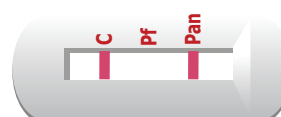
- Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen extract. Otherwise, review the whole procedure and repeat test with a new device.
- External Control:** Good Laboratory Practice recommends using external controls, positive and 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.
 - A new lot of test kit is used.
 - A new shipment of kits is used.
 - The temperature during storage of the kit falls outside of 1-30 °C.
 - The temperature of the test area falls outside of 15-30 °C.
 - To verify a higher than expected frequency of positive or negative results.
 - To investigate the cause of repeated invalid results.

INTERPRETATION OF ASSAY RESULT

- NEGATIVE RESULT:** If only the C line is present, the absence of any burgundy color in both test lines (Pan and Pf) indicates that the plasmodium antigens are not detected. The result is negative or non-reactive.



- POSITIVE RESULT:**
 - In addition to the presence of the C line, if only the Pan line develops, the test indicates the presence of pLDH antigen. The result is Pf negative or non-reactive, and positive or reactive for any of the other three *Plasmodium* species (Pv, Pm and Po) (Subject **Limitations of Test 6**).



2.2 In addition to the presence of the C line, if only the Pf line develops, the test indicates the presence of pHRP-II antigen. The result is Pf positive or reactive.



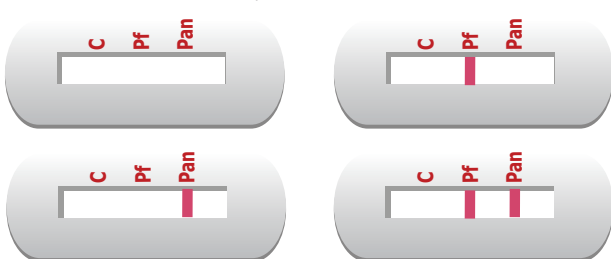
2.3 In addition to the presence of C line, if both Pan and Pf lines develop, the test indicates the presence of both pHRP-II and pLDH. The result is Pf positive or reactive. The result may also be positive or reactive for Pf and any of the other three *Plasmodium* species (Po, Pv and Pm) (Subject Limitations of Test 3).



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

3. INVALID:

If no C line develops, the assay is invalid regardless of any burgundy color in the test lines as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. Clinical Performance

1.1 Performance for Pf Ag Test

A total of 361 blood specimens were collected from a malaria endemic area (throughout India) and tested by the TRUSTline Malaria Pf/Pan Ag Rapid Test in comparison with thick blood smear test. Comparison for all the specimens is shown in the following table.

Smear Test	TRUSTline Malaria Pf/Pan Ag Rapid Test		Total
	Positive	Negative	
Positive	60	1	61
Negative	0	300	300
Total	60	301	361

Relative Sensitivity: 98.36%, Relative Specificity: 100%, Overall Agreement: 99.72%

1.2 Performance for Pan Ag Test

A total of 591 blood specimens were collected from a malaria endemic area (throughout India) and tested by the TRUSTline Malaria Pf/Pan Ag Rapid Test in comparison with thick blood smear test. Comparison for all the specimens is shown in the following table.

Smear Test	TRUSTline Malaria Pf/Pan Ag Rapid Test		Total
	Positive	Negative	
Positive	290	1	291
Negative	0	300	300
Total	290	301	591

Relative Sensitivity: 99.65%, Relative Specificity: 100%, Overall Agreement: 99.83%

1.3 External Evaluation Results

TRUSTline Malaria Pf/Pan Ag Rapid Test was externally evaluated and qualified by ICMR-NIRTH (National Institute of Research and Tribal Health), Govt. of India.

100% Sensitivity & 100% Specificity is obtained during evaluation for TRUSTline Malaria Pf/Pan Ag Rapid Test.

Qualification Criteria of recommending Malaria RDTs by ICMR-NIRTH:

For *P. falciparum*: Sensitivity and Specificity should be minimum 95% at 200 asexual parasites/μl of blood. For *P. vivax*: Sensitivity ≥ 75% at density of at 200 asexual parasites/μl of blood and Specificity should be ≥ 90%.

The qualification criteria used by NIRTH is similar to the NVBDCP, Govt. of India (National vector Borne Disease Control and Prevention). Low and High Parasites used in the study was as per WHO FIND protocol.

2. Cross-Reactivity

The negative blood specimen was spiked with serum specimens of infectious diseases and then tested according to the standard procedure. The results showed that the TRUSTline Malaria Pf/Pan Ag Rapid Test had no cross-reaction with the following tested serum specimens of infectious disease.

Specimen	Sample Size	Pf Reactivity	Pan Reactivity
Filaria Serum	10	Negative	Negative
Typhoid Serum	10	Negative	Negative
Dengue NS1 Ag Serum	10	Negative	Negative
HBsAg Serum	10	Negative	Negative
ANA Serum	10	Negative	Negative
RF (≤2,500 IU/ml)	10	Negative	Negative

3. Precision

Within run and between run precisions have been determined by testing 20 replicates with four categories of the specimens: negative, weak and strong positive specimens. The negative, weak and strong positive specimens were correctly identified in all of the tests performed in each run.

3. Interference

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

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

Potential interfering substances spiked	Pf Reactivity			Pan 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 3.48 μmol/L	-	+	+++	-	+	+++
Human IgG 150 mg/dL	-	+	+++	-	+	+++

LIMITATIONS OF TEST

- The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of plasmodium antigen in whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate test results.
- The TRUSTline Malaria Pf/Pan Ag Rapid Test is limited to the qualitative detection of plasmodium antigen in whole blood. The intensity of the test line does not have linear correlation with the antigen titer in the specimen.
- In the case that both Pan and Pf lines are visible, interpret the result cautiously. Infection by Pf alone or co-infection with Pf and any of the other three plasmodium species could result in color development on both Pan and Pf lines. Thus, when both Pan and Pf lines are visible, follow up with appropriate additional testing methods for further discrimination of plasmodium species present in the sample.
- A negative result for an individual subject indicates absence of detectable plasmodium antigen. However, a negative test result does not preclude the possibility of exposure to or infection with plasmodium.
- A negative result can occur if the quantity of the plasmodium 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.
- A positive result for pLDH and negative for pHRP-II does not necessarily rule out a Pf infection, since, due to the genetic diversity some Pf isolates lack the HRP-II gene⁸.
- Infection may progress rapidly. If the symptom persists, while the result from TRUSTline Malaria Pf/Pan Ag Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
- Some specimens containing an 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.

REFERENCES

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Index of Symbols

	Consult instructions for use		Catalogue number		Use-by date
	For <i>in vitro</i> diagnostic use only		Batch code		Tests per kit
	Temperature limit 1-30 °C		Do not re-use		Keep dry
	Manufacturer		Date of manufacture		
	If device is non-sterile		Warnings / Precautions		
	Do not use if package is damaged		Keep away from sunlight		



Athenese-Dx Pvt. Ltd.
Module No. 407 & 408, 4th Floor,
TICEL Bio Park II, No. 5, CSIR Road,
Taramani, Chennai-600113, India
Tel: +91-44-22541131
E-mail: info@athenesedx.com
Website: www.athenesedx.com

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