OnSite™ Malaria Pf/Pan Ag Rapid Test

REF R0113C CE

INTENDED USE

The *OnSite* 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.

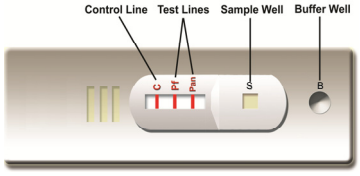
SUMMARY AND EXPLANATION OF THE TEST

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 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 performed only by well-trained microscopists using defined protocols[2], which presents major obstacles for the remote and poor areas of the world.

The *OnSite* 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 *OnSite* 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 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 antibodies 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

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. 5 µL blood transfer devices (sample loops, mini plastic droppers or capillary tubes)
3. Blood lysis buffer (REF SBR0113, 10 mL/bottle)
4. One package insert (instruction for use)

MATERIALS PROVIDED IN CERTAIN KIT CONFIGURATIONS

1. Alcohol swabs
2. Lancets or safety lancets
3. Gloves
4. Individual use blood lysis buffer

MATERIALS MAY BE REQUIRED AND AVAILABLE FOR PURCHASE

1. *Positivia* Malaria Ag Rapid Test Assay Control Kit (Cat # C0010) contains positive and negative controls

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer

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°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. Hemolyzed blood may be used for the testing, but do not use 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 bio-hazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. 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.
13. 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 2-30°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 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.

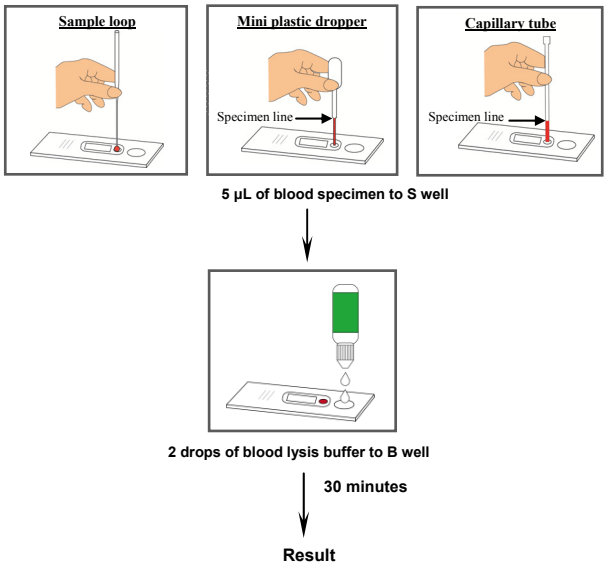
Step 3: Be sure to label the device with specimen's ID number.

Step 4: Fill the blood transfer device (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 5 µL 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 (**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

1. **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.

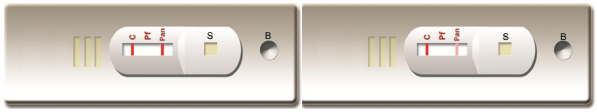
2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kit is used.
   c. A new shipment of kits is used.
   d. The temperature 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 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.

2. **POSITIVE RESULT**:

2.1 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**

Blood samples were collected from a malaria endemic area and tested by the *OnSite* Malaria Pf/Pan Ag Rapid Test and by thick blood smear test. Comparison for all subjects is shown in the following table:

	Pf		Pan	
	Positive	**Negative**	**Positive**	**Negative**
Smear test	43	280	101	99
***OnSite* Malaria Pf/Pan Ag Rapid Test**	43	280	96	104

Pf detection: Sensitivity: 100%, Specificity: 100%;
Pan detection: Sensitivity: 95%, Specificity: 100%; Kappa value: 0.98.

2. **Cross-Reactivity**

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

Antigen Concentration	Pf Reactivity	Pan Reactivity
1.0 mg/mL pHRP-II	Positive	Negative
1.0 mg/mL Pv-LDH	Negative	Positive
1.0 mg/mL Pf-LDH	Negative	Positive

Cross reaction with common microbe antigens:
A negative blood specimen was spiked with antigens from common microbes and then tested according to the standard procedure. The results showed that the *OnSite* Malaria Pf/Pan Ag Rapid Test had no cross-reaction with the following antigens at the concentration tested.

Antigen (Ag)	Concentration	Pf Reactivity	Pan Reactivity
HIV-1 p24 Ag	1.0 mg/mL	Negative	Negative
HBsAg	1.0 mg/mL	Negative	Negative
Dengue NS1 Ag (DEN1, 2, 3, 4)	1.0 mg/mL	Negative	Negative
Chikungunya virus Ag	1.0 mg/mL	Negative	Negative

Cross reactivity with specimens from other infectious disease:
No false positive Pf or Pan test results were observed on 8-19 specimens from the following disease states or special conditions:

HAV	HBV	HCV	HIV	*H. pylori*
Dengue	TB	*T. pallidum*	ANA	HAMA
RF (up to 2,500 IU/mL)				

3. **Interference**

Common substances (such as pain and fever medication, blood components) may affect the performance of the *OnSite* 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 controls. The results demonstrate, at the concentrations tested, the substances studied didn't affect the performance of the *OnSite* Malaria Pf/Pan Ag Rapid Test.

List of potentially interfering substances and concentrations tested:

1. Albumin	60 g/L	6. Glucose	5 mmol/L
2. Bilirubin	20 mg/dL	7. Human IgG	0 mg/dL
3. Creatinine	442 µmol/L	8. Hepatitis	0 U/L
4. EDTA	3.4 µmol/L	9. Salicylic acid	4.5 mmol/L

LIMITATIONS OF TEST

1. 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.
2. The *OnSite* 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.
3. **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 results 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.**
4. 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.
5. 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.
6. A result positive 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 gene[7,8].
7. Infection may progress rapidly. If the symptom persists, while the result from *OnSite* Malaria Pf/Pan Ag Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
8. Some specimens containing an unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
9. 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 CE Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	N	Tests per kit
2°C 30°C	Store between 2-30°C	EC REP	Authorized Representative	2	Do not reuse
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
10110 Mesa Rim Road
San Diego, CA 92121, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany

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English version

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