OnSite™ Malaria Pf/Pv Ab Combo Rapid Test

REF R0111C CE

INTENDED USE

The *OnSite* Malaria Pf/Pv Ab Combo Rapid Test is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of antibodies including IgG, IgM and IgA to *Plasmodium falciparum* (*Pf*) and *Plasmodium vivax (Pv)* in human serum, plasma or whole blood. It 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 *P. falciparum* and *P. vivax*.

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.

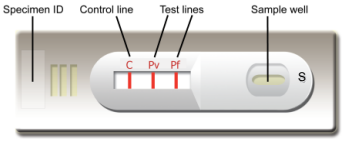
SUMMARY AND EXPLANATION OF THE TEST

Malaria is a mosquito-borne, hemolytic, febrile illness that affects over 200 million people and kills more than half a million people per year[1]. Malaria is caused by four species of the protozoan parasite *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[2].

Traditionally, malaria is diagnosed by the demonstration of the organisms in Giemsa stained smears of peripheral blood, and the different species of *Plasmodium* are distinguished by their appearance in infected erythrocytes[2]. This technique is performed only by well-trained microscopists using defined protocols, which presents major obstacles for remote and underprivileged areas of the world[3,4].

The *OnSite* Malaria Pf/Pv Ab Combo Rapid Test detects antibodies present in serum, plasma or whole blood that are generated in response to the infection with *P. falciparum* and *P. vivax*[5]. Utilizing the highly reactive *Pf* specific antigen, *Pf*-MSP, and the *Pv* specific antigen, *Pv*-MSP, the test enables simultaneous detection and differentiation of infections with *P. falciparum* and *P. vivax*. The test can be performed within 15-20 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* Malaria Pf/Pv Ab Combo Rapid Test is a double antigen lateral flow chromatographic immunoassay. The strip in the test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant *Pf*-MSP and *Pv*-MSP antigens conjugated with colloidal gold (*Pf* conjugates and *Pv* conjugates) and a control antibody conjugated with colloidal gold and 2) a nitrocellulose membrane strip containing two test lines (Pv and Pf lines) and a control line (C line). The Pf line is pre-coated with recombinant *Pf*-MSP antigen for the detection of antibodies to *Pf*. The Pv line is pre-coated with *Pv*-MSP antigen for the detection of antibodies to *Pv*. The C line is pre-coated with a control line antibody.

When an adequate volume of test specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. Antibodies (IgG, IgM and IgA) to *P. falciparum or P.vivax*, if present in the specimen, will bind to the *Pf* or the *Pv* conjugates, respectively. The immunocomplexes are then captured on the membrane by either the pre-coated *Pf*-MSP or the *Pv*-MSP antigens forming a burgundy colored Pf line (*Pf* positive result) and/or Pv line (*Pv* positive result).

Absence of any test lines suggests a negative result. The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex or the control antibody regardless of color development on any of the test lines. If the C line does not develop, the 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. Two-mark capillary tubes (10/20 µL)
3. Sample diluent (REF SB-R0111C, 5 mL/bottle)
4. One package insert (instruction for use)

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

1. Positive Control
2. Negative Control

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. Lancing device for whole blood test

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 may lead to 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 components from any other type of kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimens for testing.
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 controls in the same manner as the patient specimens.
12. The test result should be read 15-20 minutes after a specimen is applied to the sample well or sample pad of the device. Any results interpreted outside 15-20 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 devices 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 using standard bio-safety procedures.

Plasma

Step 1: Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by venipuncture.
Step 2: Separate the plasma by centrifugation.
Step 3: Carefully withdraw the plasma into a new pre-labeled tube.

Serum

Step 1: Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by venipuncture.
Step 2: Allow the blood to clot.
Step 3: Separate the serum by centrifugation.
Step 4: Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. If not tested immediately, store specimens at 2-8°C for up to 5 days. For longer storage, specimens should be frozen at -20°C.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Whole Blood

Drops of whole blood can be obtained by different methods, such as venipuncture. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®).Do not use clotted blood for testing.

The whole blood specimens must be tested within 24 hours of collection. The specimens should be stored in refrigeration (2-8°C) if not tested immediately.

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.

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

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

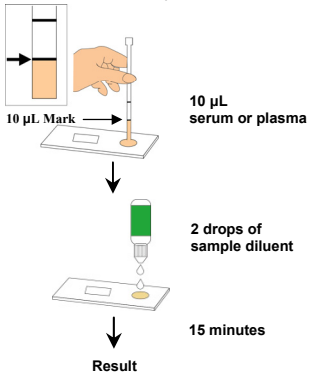
Step 4: **The volume of the specimen is different according to different type of the specimen.**

FOR SERUM, PLASMA SPECIMENS (10 µL)

4.1 Fill the capillary tube with serum or plasma not to exceed the specimen mark (**10 µL Mark**) as shown in the following image. The volume of the specimen is around 10 µL.

4.2 Holding the capillary tube vertically, dispense the entire amount of specimen into the center of the sample well making sure that there are no air bubbles. **For better precision, transfer specimen using a pipette capable of delivering a volume of 10 µL for serum or plasma.**

4.3 Immediately add 2 drops (60-80 µL) of sample diluent to the sample well with the bottle positioned vertically.

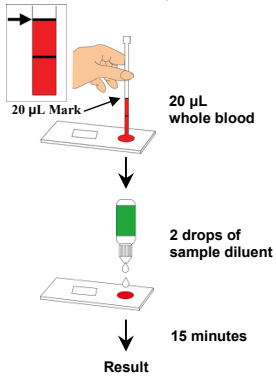


FOR WHOLE BLOOD SPECIMENS (20 µL)

4.1 Fill the capillary tube with whole blood not to exceed the specimen mark (**20 µL Mark**) as shown in the following image. The volume of the specimen is around 20 µL.

4.2 Holding the capillary tube vertically, dispense the entire amount of specimen into the center of the sample well making sure that there are no air bubbles. **For better precision, transfer specimen using a pipette capable of delivering a volume of 20 µL for whole blood.**

4.3 Immediately add 2 drops (60-80 µL) of sample diluent to the sample well with the bottle positioned vertically.



Step 5: Set up timer.

Step 6: Read results at 15-20 minutes. Positive results may be visible in as short as 1 minute. Negative results must be confirmed at the end of the 20 minutes only. ***However, any results interpreted outside 15-20 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

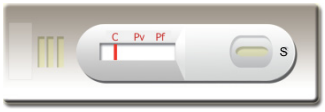
1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen and sample diluent. If the C line does not develop, review the entire procedure and repeat the test with a new device.

2. **External Control:** Good Laboratory Practice recommends using external positive and negative controls to ensure 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 kits is used.
c. A new shipment of test 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 (*Pf* and *Pv*) indicates that antibodies to *Pf* and *Pv* are not detected in the specimen. The result is negative or non-reactive for both *Pf* and *Pv*.



2. **POSITIVE RESULT**:

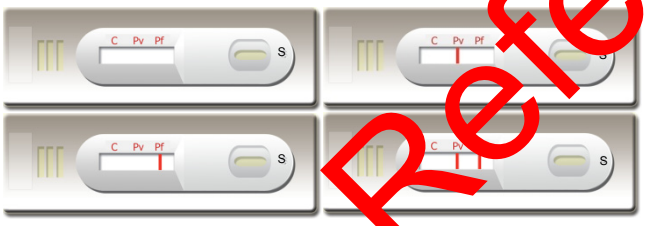
2.1 In addition to the presence of the C line, if only the Pv line develops, the test result indicates the presence of antibodies to *Pv* in the specimen. The result is *Pv* positive or reactive and *Pf* negative or non-reactive.

2.2 In addition to the presence of the C line, if only the Pf line develops, the test result indicates the presence of antibodies to *Pf* in the specimen. The result is *Pf* positive or reactive and *Pv* negative or non-reactive.

2.3 In addition to the presence of the C line, if both Pf and Pv lines develop, the test result indicates the presence of antibodies to *Pf and Pv* in the specimen. The result is *Pf and Pv* positive or reactive.

Specimens 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. **Performance for *Pf* Ab Test**

A total of 195 samples were collected from susceptible subjects and tested with the *OnSite* Malaria Pf/Pv Ab Combo Rapid Test and with a commercial Malaria Pf/Pv Ab Combo Rapid Test on the market. Comparison for all subjects is shown in the following table.

	OnSite Malaria Pf/Pv Ab Combo Rapid Test		
Reference	**Positive**	**Negative**	**Total**
Positive	26	2	28
Negative	2	165	167
Total	28	167	195

Relative Sensitivity: 92.9%, Relative Specificity: 98.8%, Overall Agreement: 97.9%

2. **Performance for *Pv* Ab Test**

A total of 196 samples were collected from susceptible subjects and tested with the *OnSite* Malaria Pf/Pv Ab Combo Rapid Test and with a commercial Malaria Pf/Pv Ab Combo Rapid Test. Comparison for all subjects is shown in the following table.

	OnSite Malaria Pf/Pv Ab Combo Rapid Test		
Reference	**Positive**	**Negative**	**Total**
Positive	66	2	68
Negative	5	123	128
Total	71	125	196

Relative Sensitivity: 97.1%, Relative Specificity: 96.1%, Overall Agreement: 96.4%

3. **Cross-Reactivity**

No false positive Pf or Pv test results were observed on 4-10 specimens from the following disease states or special conditions:

HAV	HBV	HCV	HEV	HIV
hCG	Dengue	*H. pylori*	TB	*T. pallidum*
Typhoid	ANA	HAMA	RF (up to 8,400 IU/mL)	

4. **Interference**

Common substances (such as pain and fever medication and blood components) may affect the performance of the *OnSite* Malaria Pf/Pv Ab Combo Rapid Test. This was studied by spiking these substances into three levels of *Pf* Ab and *Pv* Ab standard controls (negative, weak positive and strong positive). The results demonstrate, at the concentrations tested, the substances studied do not affect the performance of the *OnSite* Malaria Pf/Pv Ab Combo Rapid Test.

List of potentially interfering substances and concentrations tested:

1. Albumin	60 g/L	6. Human IgG	150 mg/dL
2. Bilirubin	20 mg/dL	7. Hemoglobin	2 g/L
3. Creatinine	442 µmol/L	8. Heparin	3,000 U/L
4. EDTA	3.4 µmol/L	9. Salicylic acid	4.34 mmol/L
5. Glucose	5.5 mmol/L	10. Sodium citrate	3.8%

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result sections must be followed closely when testing for the presence of antibodies to *P. falciparum* and *P. vivax* parasites in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may lead to inaccurate test results.
2. The *OnSite* Malaria Pf/Pv Ab Combo Rapid Test is limited to the qualitative detection of antibodies to *P. falciparum* and *P. vivax* parasites in human serum, plasma or whole blood. The intensities of the test lines do not have linear correlation with the antibody titers in the specimen.
3. A negative or non-reactive result for an individual subject indicates absence of detectable anti-*P. falciparum* and *P. vivax* antibodies. However, a negative test result does not preclude the possibility of exposure to or infection with *Plasmodium* parasites.
4. A negative or non-reactive result can occur if the quantity of the anti-*P. falciparum* and *P. vivax* antibodies present in the specimen is below the detection limits of the assay or the antibodies that are detected are not present during the stage of the disease in which a sample is collected.
5. Infection may progress rapidly. If the symptoms persists, while the result from *OnSite* Malaria Pf/Pv Ab Combo Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
6. Some specimens containing unusually high titers 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

1. World Malaria Report 2011. World Health Organization. http://www.who.int/malaria/world_malaria_report_2011/en
2. Malaria, p. 421-424. Chapter 9. Infectious and Parasitic Diseases. Rubin E., Farber JL: Pathology, 2nd ed. 1994. J.B. Lippincott, Philadelphia.
3. Cook GH, Chiodini PL, Doherty T, et al, Am J Trop Med. Hyp, 1999, Feb: 60(2):173-2.
4. Arch Pathol Lab Med. 2013 Jun;137(6):805-11.
Miller LD, Burgess DC, Taylor HJ, Kain KC. Bull World Health Organ. 1999;77(7):553-9.

Index of CE Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	Σ 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

PI-R0111C Rev. E
Effective Date: 2015-11-25
English Version

For Export Only, Not For Re-sale In the USA