MALARIA MBPan

For *in Vitro* diagnostic use only

Immunochromatographic test for detection of Malaria P.f/P.v/P.o/P.m in human blood specimen

I. INTENDED USE

Malaria MBPan 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.

II. SUMMARY AND EXPLANATION OF THE TEST

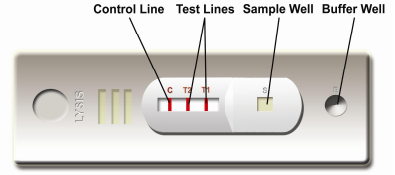
Malaria is a mosquito-borne, hemolytic, febrile illness that infects over 200 million people and kills more than 1 million people per year. It is caused by four species of *Plasmodium*: *P. falciparum, P. vivax, P. ovale, and P. malariae*. These plasmodia all infect and destroy human erythrocytes, producing chills, fever, anemia, and splenomegaly. *P. falciparum* causes more sever 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[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 capable of accurate and reliable diagnosis, but only when performed by skilled microscopists using defined protocols[2], which presents major obstacles for the remote and poor areas of the world.

The Malaria MBPan 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 plasmodia[3-6]. It can be performed by untrained or minimally skilled personnel, without laboratory equipment.

III. TEST PRINCIPLE

The Malaria MBPan 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 colloid gold (pHRP II-gold conjugates) and mouse anti-pLDH antibody conjugated with colloid 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.

IV. COMPONENTS

Each kit contains everything needed to perform 30 test (REF VQ81706):

1) Test device
2) Blood Lysis buffer
3) 5 µL mini plastic droppers.
4) Instruction for use

V. STORAGE AND STABILITY

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.

VI. WARNING AND PRECAUTIONS

1) For in vitro diagnostic use and professional use only.
2) Read the package insert instruction before use the kit.
3) Do not use beyond the expiration date which appears on the package label.
4) Do not open the sealed pouch, unless ready to conduct the assay.
5) Bring all reagents to room temperature (15°C-30°C) before use.
6) Do not use the components in any other type of test kit as a substitute for the components in this kit.
7) Haemolized blood may be used for the testing, but do not take precipitants.
8) Wear protective clothing and disposable gloves while assaying samples. Wash hands thoroughly after performing the test.
9) Handle all specimens as if they contain infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving them for at least one hour. Alternatively, they can be treated with 0.5 to 1% solution of sodium hypochlorite for one hour before disposal.

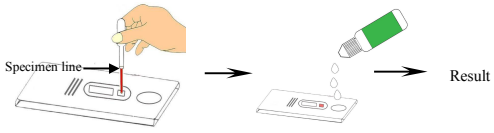
10) Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
11) Handle the Negative and Positive Control in the same manner as patient specimens.
12) The testing results should be read within 30 minutes after a specimen is applied to the sample well or sample pad of the 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.
14) As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
15) Excess sample volume (>5µL) can give false positives

VII. SPECIMEN COLLECTION AND STORAGE

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.

VIII. TEST PROCEDURE

1. Allow samples and reagents to come to room temperature prior to testing if refrigerated or frozen. Mix the specimen well prior to assay once thawed. Blood will be haemolyzed after thawing.
2. Remove the «reaction device» from its protective wrapper. Place the test device on a clean, flat surface.
3. Label device with the patient's name or control number.
4. Fill in the mini plastic dropper with the blood specimen not to exceed the specimen line as showed in the following image (about 5 µL). Holding the dropper vertically, dispense all of the specimen into the center of the sample well making sure that there are no air bubbles.



5 µL of blood specimen **3 drops of lysis buffer** **20-30 minutes**

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 5µL of volume.

5. Add 3 drops (100-150 µl) of Lysis Buffer immediately in developer well.
6. Read the test result in 20 to 30 min. It may take more than 20 minutes to have the background become clearer.

IX. INTERPRETATION OF RESULTS

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.

POSITIVE RESULT:
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.**

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.**

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.

X. PERFORMANCE CHARACTERISTICS

1. Clinical Performance with Pf positive specimen

A total of 224 samples from susceptible subjects were tested by the Malaria MBPan and by thick blood smear test. Comparison for all subjects is showed in the following table.

	Malaria MBPan		
Smear test	**Positive**	**Negative**	**Total**
Positive	24	0	24
Negative	3	197	200
Total	27	197	224

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

2. Clinical Performance with Pv positive specimen

A total of 224 samples from susceptible subjects were tested by the Malaria MBPan and by thick blood smear test. Comparison for all subjects is showed in the following table.

	Malaria MBPan		
Smear test	**Positive**	**Negative**	**Total**
Positive	9	0	9
Negative	3	212	215
Total	12	212	224

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

3. Specificity

The study was conducted with 3 separated lots of the Malaria MBPan with Serum, plasma or whole blood to determine the Specificity: Serum with trygliceride concentration up to 500 mg/ml, Serum with Bilirubin concentration up to 10 mg/100ml, Haemolized specimens with haemoglobin concentration up to 10 mg/ml, Prostatic acid phosphatase with concentration up to 1000 ng/ml, albumin with concentration up to 20 mg/ml.
Conclusion: All of the above were analyzed and did not show interference or cross reactivity with the test.

XI. LIMITATION OF PROCEDURE

1. 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.
2. The Malaria MBPan 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. **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.**
4. 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.
5. 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.
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.

XII. BIBLIOGRAPHY

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CONTENTS	**Ref. VQ81706 (30 test)**
Test Device	30 items
Blood Lysis buffer	1 x 10 mL
5 µL mini plastic droppers.	30 items
Instruction for use	1 item

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer
	Consult Instructions for Use		Use By (year/month)	REF	Catalogue number		Do not reuse
	Keep dry		Fragile, handle with care	NON STERILE	Non-sterile		Keep away from heat

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