INSTRUCTIONS FOR USE

MALARIA MBPAN

RAPID IMMUNOCHROMATOGRAPHIC TEST FOR DETECTION OF *MALARIA P.F/P.V/P.O/P.M* IN HUMAN BLOOD SPECIMEN

1 – INTRODUCTION AND INTENDED USE

For ***in Vitro*** diagnostic use only

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 episodes, anemia, and splenomegaly. *P. falciparum* causes more sever 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].

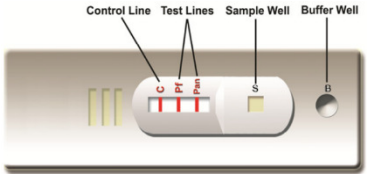
Traditionally, malaria is diagnosed by the demonstration of the organisms on Giemsa stained thick smears of peripheral blood, and the different species of *Plasmodium* are distinguished by their appearance in infected erythrocytes[1]. The technique is performed only by well-trained microscopists using defined protocols[2], which presents major obstacles for the remote and poor areas of the world.

The Malaria MBPan 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.

Malaria MBPan is a manual 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.

2 - PRINCIPLE OF THE METHOD

The Malaria MBPan is a lateral flow chromatographic immunoassay. The test strip components consist of: 1) a 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 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 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 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.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
MALARIA MBPan CND: W0104050202; EDMA: 14.05.02.02; RDM: 2147497/R	Immunochromatographic test	**VQ81706** (30 tests)	30 sealed in foil pouch containing the device, with dessicant. 1 plastic tube with dropper tip containing the blood Lysis buffer. (1 x 10 mL). 5 µL blood transfer devices – mini plastic dropper. (30 items) Secondary packaging: cardboard box.

4 - MATERIALS REQUIRED BUT NOT PROVIDED

Disposable gloves, Timer.

5 - PRECAUTIONS AND WARNINGS

- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- MALARIA MBPan is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by qualified laboratory personnel also minimally skilled.
- This product is not classified as dangerous according to current European legislation.
- Read the package insert instruction before use the kit.
- Avoid touching the nitrocellulose with your fingers.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- Each test device is for single use only.
- Never use reagents from another lot. Do not use the components in any other type of test kit as a substitute for the components in this kit.
- The test should remain in the sealed pouch until use.
- Do not use the test if pouch is damaged.
- Haemolized blood may be used for the testing, but do not take precipitants.
- Wear gloves when handling the sample.
- Disposable gloves, lysis buffer, mini plastic dropper, and used devices in a propre biohazard container.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- The reagents' quality cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.

- The testing results should be read within 30 minutes after a specimen is applied to the sample well of the device. Read result after 30 minutes should be considered invalid and must be repeated.
- Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.masciabrunelli.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.
- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device. complaint@masciabrunelli.it
- 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.

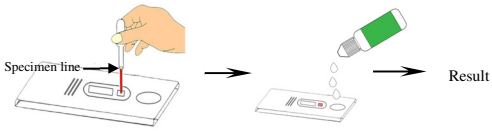
6 - Storage conditions and specimen collection

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 30°C. Do not freeze the kit. 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.
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.

7 - Test procedure

Allow the tests, samples and reagent to reach to room temperature (15-30ºC/59-86ºF) prior to testing. Do not open pouches until ready to perform the assay.

1. 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 **(S well)** making sure that there are no air bubbles.



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 2 drops (50-100 µl) of Lysis Buffer immediately in developer well **(B well)**.
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.**

8 – Reading and interpretation of results

NEGATIVE RESULT: If only the C line is present, the absence of any 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 C line, if only the Pan line develops, the test indicates for 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).**

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

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 positive determination is made.

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

9 - Internal quality control

The Internal Quality Control procedure is included in each test strip. A line appearing in the control region (C) is an internal control. It confirms sufficient specimen volume and correct procedural technique.

10 - Performances characteristics

1. Clinical Performance with Pf positive specimen

Blood samples were collected from a malaria endemic area and tested by the Malaria MBPan and by thick blood smear test. Comparison for all subjects is showed in the following table.

	Pf		Pan	
	Positive	Negative	Positive	Negative
Smear test	43	280	101	99
Malaria MBPan	43	280	96	104

Pf detection:
Sensitivity: 100%, Specificity: 100%;
Pan detection:
Sensitivity: 95%, Specificity: 100%, K 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 Malaria MBPan, 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 Malaria MBPan 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 T. pallidum ANA HAMA RF (up to 2.500 IU/mL) TB

3. Interference

Common substances (such as pain and fever medication, blood components) may affect the performance of the Malaria MBPan. This was studies 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 Malaria MBPan.

List of potentially interfering substances and concentrations tested:

Albumin	60 g/L	Creatinine	442 µmol/L	Glucose	55 mmol/L	Human IgG	150 mg/dL
Bilirubin	20mg/dL	EDTA	3,4 µmol/L	Heparin	3.000 U/L	Salicylic acid	4,34 mmol/L

11 - LIMITATIONS OF THE TEST

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of plasmodium antigen in whole blood from individual subjects. Failure to follow the procedure may give inaccurate test results.
2. The Malaria MBPan 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 antigen 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 persist, while the result from Malaria MBPan is negative or non-reactive, it is recommended to test with an alternative test method.
8. Some specimens containing 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.

12 - REFERENCES

1. Malaria, p. 421-424. Chapter 9. Infectious and Parasitic Diseases. Rubin E., Farber JL: Pathology, 2nd ed. 1994. J.B. Lippincott, Philadelphia.
2. Cooke AH, Chiodini PL, Doherty T, et al, Am J Trop Med. Hyp, 1999, Feb: 60(2):173-2
3. Guthmann JP, et al: Trans R Soc Trop Med Hyg. 2002, 96(3):254-7
4. Kar I, Eapen A, Adak T, Sharma VP, Indian J Malariol. 1998, 35(3):160- 2
5. Mills CD, Burgess DC, Taylor HJ, Kain KC. Bull World Health Organ. 1999;77(7):553-9
6. Cloonan N, Fischer K, Cheng Q, Saul A. Mol Biochem Parasitol. 113(2):327-30
7. Gamboa D, Ho M.F., Bendezu J, et al. PLOS One, 2010, 5(1), e8091.
8. Koita OA, Doumbo OK, Quattara A, et al. Am J Trop Med Hyg. 2012 Feb;86(2): 194-8.

TABLE OF APPLICABLE SYMBOLS

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (DXXX)		Manufacturer		Keep dry	UDI	Unique device identifier
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content	2023/01

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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