

## IMMUNOQUICK® MALARIA +4

Rapid test for detection of *Plasmodium falciparum* and 3 other species (*Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium ovale*) in blood.

### INTENDED USE

IMMUNOQUICK® MALARIA +4 is a simple and rapid self-performing, qualitative immunoassay test for the detection of the in vitro presence of *Plasmodium falciparum* specific histidine rich protein-2 (Pf HRP-2) and pan malaria specific pLDH (pan lactate dehydrogenase) in human blood. It can be used on general population including neonates and pregnant women. The test can be used in patients with symptoms of malarial infection for the specific detection of *P. falciparum* and differentiation of others malarial species and for the follow up of antimalarial therapy.

IMMUNOQUICK® MALARIA +4 is intended for a use on whole blood and does not require any instrumentation or dedicated facilities. The test is intended for use by health care professionals in clinical laboratories or on the point of care.

### TEST PRINCIPLE

A pair of monoclonal antibodies anti-HRP-2 is used for the HRP-2 detection. One is immobilized on the nitrocellulose membrane at the level of the HRP-II test line: it corresponds to the capture antibody. Another one is labelled with colloidal gold for the subsequent revelation.

A similar principle is used for the pLDH detection with a pair of monoclonal antibodies anti-pLDH, the capture antibody being immobilised at the level of the pLDH test line, and the other one being labelled with colloidal gold for the subsequent revelation. During the sample migration, HRP-2 and pLDH, if present in the sample, will form antigen-antibody complexes with the labelled antibodies. These complexes will be captured by the capture antibodies on the HRP-II and pLDH test lines, creating one or two purple coloured lines generated by gold nanoparticles.

The presence of a purple internal control line in the upper part of the membrane (control line) indicates that the result is valid and that the followed procedure is correct.

### MATERIAL PROVIDED

- Strips in individual pouch
- Lysis buffer
- Patients cards
- Tubes
- Instructions for use
- Rack

### MATERIAL REQUIRED BUT NOT PROVIDED

- Stop watch with alarm
- Laboratory pipettes (optional)
- Sterile lancets (optional)
- Desinfectant tissues (optional)

### STORAGE AND STABILITY

- The kit can be preserved at room temperature or cooled (2-30°C).
- The strips are stable until the expiry date indicated on the aluminium pouch. It must be stored in the pouch only. Not to use beyond the expiry date.
- The dropper vial has to be closed after each use. Replace the dropper vial of lysis buffer in the kit after each use. The opened vial of lysis buffer is stable until the expiry date indicated on the vial.

### PRECAUTIONS

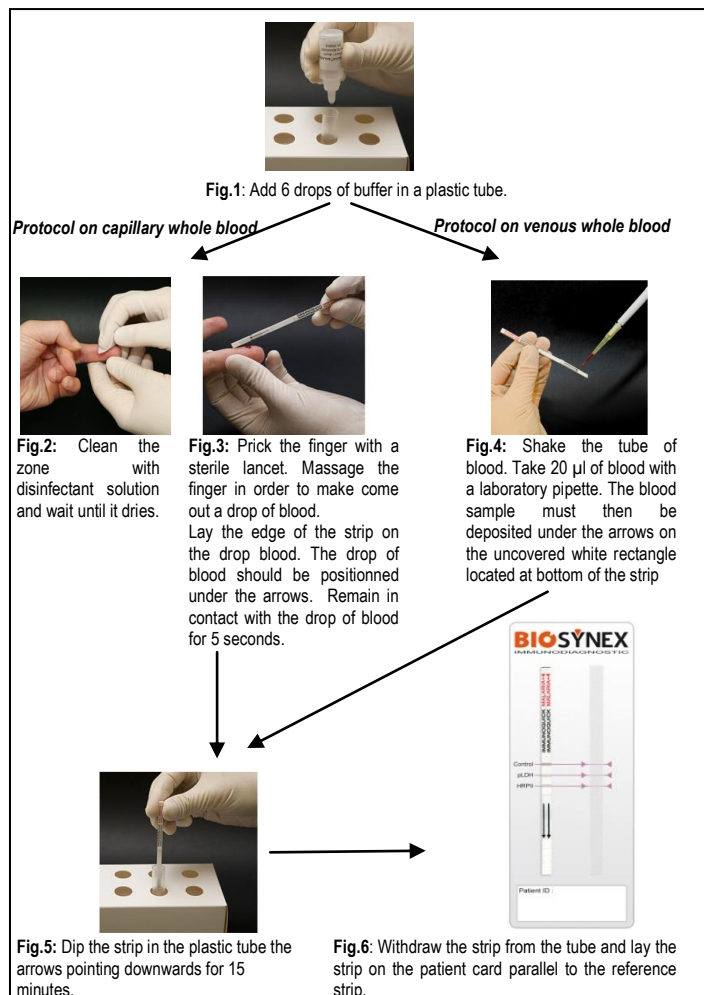
- For diagnostic in vitro use only. Not to use after the expiry date.
- Not to eat, drink or smoke during the handling of the samples and the test.
- Not pipette with the mouth.
- The samples of blood must be regarded as potentially infectious. When realizing the test, necessary precautions for handling of infectious products should be taken. The various elements of the test and the samples should be proceeded according to the procedure reserved to potentially infectious waste.
- Wear lab coat, gloves and ocular protections when performing the test.
- Clean the hands after realization of the test.
- Not to interchange the reagents of various batches.
- Each test is intended for single use only.
- Do not use a strip once aluminium pouch has been opened or damaged

### SPECIMEN COLLECTION AND HANDLING

Capillary or venous blood can be used. Clean the skin with a disinfectant solution and wait until the zone becomes dry. In the case of collection of venous blood, heparinised, oxalate or EDTA tubes can be used because none of these anticoagulants interacts with the test. In case the test cannot be run immediately,

whole blood can be stored a maximum of 72 hours at 2-8°C or frozen at -70 ° C for several years.

### PROCEDURE



1. Let return components of the kit at room temperature. In the event of conservation at 2-8°C, wait 30 minutes for return to room temperature.
2. Open the pouch right before use, Take out the strip.
3. Add 6 drops of buffer in a plastic tube (Fig. 1).
4. **a. Protocol on capillary whole blood**
  - Select the zone of puncture (usually the side of 3rd or the 4th finger). Clean the zone with a disinfectant solution and wait until it dries (Fig. 2).
  - Prick the finger with a sterile lancet. Massage the finger in order to make come out a drop of blood.
  - Lay the edge of the strip on the drop of blood; the drop of blood should be positioned under the arrows. Remain in contact with the drop of blood for approximately 5 seconds. (Fig. 3).
4. **b. Protocol on venous whole blood in tube**

When venous blood is used, agitate the tube of blood before taking the test specimen. Take 20 µl of blood with a laboratory pipette. The blood sample must then be deposited under the arrows on the uncovered white rectangle located at bottom of the strip (Fig. 4).

Note: It is possible to proceed with microvolumes of 5 µL especially in children.
5. Dip the strip in the tube the arrows pointing downwards (Fig.5). Keep the strip in the tube in vertical position for 15 minutes.

- Withdraw the strip from the tube and lay the strip on the patient card parallel to the reference strip (Fig.6).
- Read the result between 15 minutes and 30 minutes from the time the strip is dipped in the tube. A negative pLDH result can only be confirmed after a reading at 30 minutes. Not to read and interpret the result beyond the 30th minute.
- After the reading, eliminate IMMUNOQUICK® Malaria +4 strip and tube according to the procedure reserved for potentially infectious waste.

## RESULTS

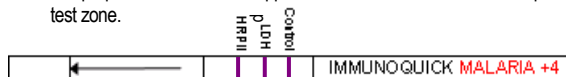
A proper reading requires:

- A minimum visual acuity
- Correct lighting conditions

### POSITIVE for *Plasmodium falciparum* and/or mixed infection:

Presence of three distinct bands:

- A purple control band must appear at the level of the control zone, and
- two purple test bands must appear at the level of the HRP II and pLDH regions of the test zone.



The result is positive even if the intensity of the purple test band T is very weak. The colored test band T may appear before the 15th minute in case of strong positive samples. In case of low positive sample, the Test band may be weak at 15 minute and it is recommended to read again at 30 minutes to get a stronger signal (especially for pLDH band).

### POSITIVE for other species:

Presence of two distinct bands:

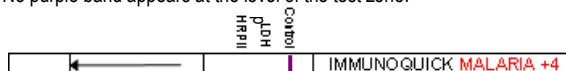
- A purple control band must appear at the level of the control zone, and
- a purple test band must appear at the level of pLDH region of the test zone.



### NEGATIVE:

Only one purple band appears at the level of the control zone.

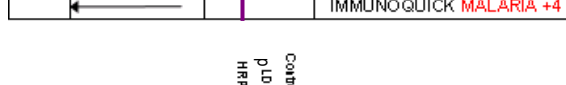
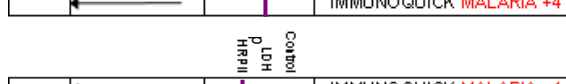
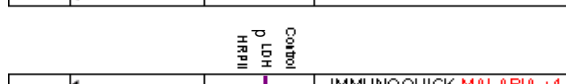
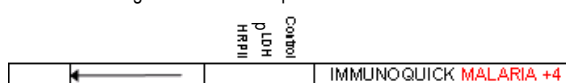
No purple band appears at the level of the test zone.



### INVALID:

Absence of purple control band.

The procedure was not followed correctly or the test was deteriorated. Do not interpret the result. Test again with a new strip.



## QUALITY CONTROL

- Internal procedural controls are included in the test. A colored line appearing in the control zone (C) ensures that sufficient specimen volume has been loaded and that the correct procedure has been followed by the operator.
- A red colour along the membrane indicates a satisfactory lysis of erythrocytes. In case of a white background on the membrane after migration, the result is invalid. The test should be repeated with another strip.

## LIMITATIONS OF PROCEDURE

- As with all diagnostic tests, the test result must always be correlated with clinical findings.
- The results of test are to be interpreted within the epidemiological, clinical and therapeutic context. When it seems indicated, the parasitological techniques of

reference should be considered (microscopic examination of the thick smear and thin blood films).

- Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
- The strips and buffer of different lots must not be mixed and used.
- In case of infection due to *P.falciparum*, or due to mixed infection, the pLDH malaria band will also be positive. Hence differentiation of infection due to *P. malariae*, *P.ovale* or *P.vivax* cannot be done.
- While monitoring therapy, if the reaction of the test remains positive with the same intensity after 5-10 days, post treatment, the possibility of a resistant strain of malaria has to be considered.
- Usually, the pLDH band turn negative after successful anti malarial therapy. However, since treatment duration and medication used affect the clearance of parasites, the test should be repeated after 5-10 days of start of treatment.
- In *P. falciparum* malaria infection, HRP-2 is not secreted in gametogony stage. Hence, in "healthy carriers", the HRP-2 band may be absent.
- HRP-2 levels, post treatment persists upto 15 days. So the pLDH band can be used to monitor success of therapy, in *P. falciparum* malaria cases.
- In a few cases, where the HRP-2 band is positive and the pLDH band is negative, it may indicate a case of post treatment malaria. However, such a reaction pattern may also be obtained in a few cases of untreated malaria. Retesting after 2 days is advised, in such cases.
- In some infections especially *Plasmodium ovale* infections, the pLDH band is missing.

## PERFORMANCES

An hospital evaluation was run comparatively to microscopic examination of thick (Giemsa) and thin (Diff-quick) blood smears as per WHO standards (40000 RBC for thin smear and 1000 WBC for thick smear). Results are summarized in table hereunder:

### HRPII line

Total samples	Results	Negative	Positive
85	Positive for <i>Plasmodium falciparum</i>	1	84
73	Positive for others <i>Plasmodium</i>	73	0
72	Absence of <i>Plasmodium</i>	72	0

### pLDH line

Total samples	Results of thin and thick smear	Negative	Positive
85	Positive for <i>Plasmodium falciparum</i>	17	68
34	Positive for <i>Plasmodium vivax</i>	9	23
8	Positive for <i>Plasmodium malariae</i>	2	6
31	Positive for <i>Plasmodium ovale</i>	18	13
72	Absence of <i>Plasmodium</i>	72	0

	pLDH	HRPII
<b>Sensibility</b>	110/156 = 70.5%	84/85 = 98.8%
<b>Specificity</b>	72/72 = 100%	145/145 = 100%

## BIBLIOGRAPHY

- Howard, R. J. et al 1986: Secretion of a Malarial Histidine-rich Protein (Pf HRP II) from *Plasmodium falciparum*-infected Erythrocytes. J. cell Biol 103,1269-1277.
- Rock, E.P. et al 1987: Comparative Analysis of the plasmodium falciparum Histidine-Rich Proteins HRP-I HRP-II and HRP-III in Malaria Parasites of Diverse Origin. Parasitol, 95,209-227.
- Parra, M.E. et al 1991: Identification of *Plasmodium falciparum* Histidine-Rich Protein 2 in the Plasma of Humans with Malaria. J. Clin. Microbial. 29,1629-1634.
- Rodriguez-Del Valle, M. Et al 1991: Detection of Antigens and Antibodies in the Urine of Humans with *Plasmodium falciparum* Malaria J. Clin Microbial. 29 1236-1242.

## SYMBOLS

	Attention, see instructions for use		Lot number
	For <i>in vitro</i> diagnostic use only		Manufacturer
	Store between 2-30°C		Do not reuse
	Tests per kit		Catalog number
	Expiry		

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