

IMMUNOQUICK® MALARIA *falciparum*

Rapid test for detection of *Plasmodium falciparum* in blood.

INTENDED USE

IMMUNOQUICK® MALARIA *falciparum* is a simple and rapid immunoassay test making it possible to detect the in vitro presence of *Plasmodium falciparum* in blood. It can be used on general population including neonates and pregnant women. The test uses a system of capture of the specific soluble antigen of *Plasmodium falciparum*: "Histidin rich protein II" (PfHRP-II) which is present and diffuse from infected red blood cells.

IMMUNOQUICK® MALARIA *falciparum* 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.

During the sample migration, HRP-2, 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 test line, creating one purple coloured line 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 packed in individual pouches
- Tubes
- Lysis buffer
- Instruction for use
- Patient cards
- Tube rack

MATERIAL REQUIRED BUT NOT PROVIDED

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

STORAGE AND STABILITY

- The kit can be stored at room or cooled temperature (2-30°C). The strips are stable until the expiry date indicated on the aluminium pouch. It must be stored in the pouch only.
- 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.

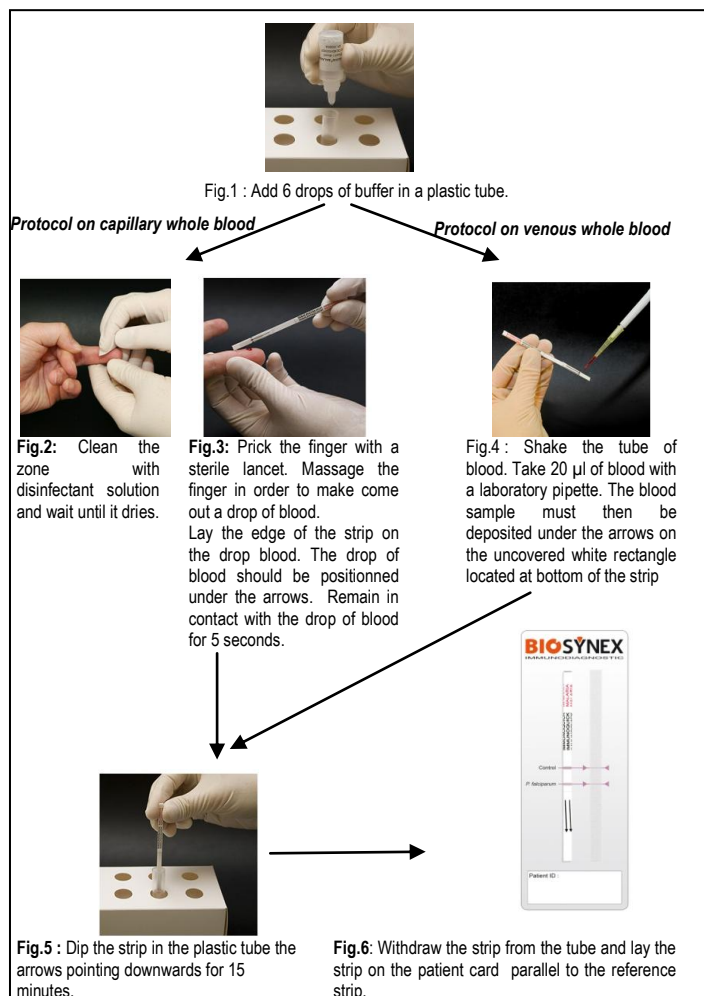
PRECAUTIONS

- 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 pipeting 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 a 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, heparinized, 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 storage at 4°C, wait 30 minutes for return to room temperature.
2. Open the aluminium pouch right before use and take out the strip.
3. Add 6 drops of buffer in a plastic tube (Fig. 1).
4. **a. Protocol on capillary 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 blood**
 - When venous blood is used, agitate the tube before taking the test specimen. Take 20µl of blood with a laboratory pipette. The blood sample must then be dropped 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). Gently hit the dipstick on the bottom of the tube to enhance migration. Keep the strip in the tube in vertical position for at least 10 minutes.
6. Withdraw the strip from the tube and lay the strip on shaded zone of the patient card parallel to the reference strip (Fig.6)

7. Read the result between 10 and 15 minutes from the time the strip is dipped in the tube. **Important:** The test result is only valid up to 15 minutes following the time the strip is dipped in the tube.
8. After the reading, eliminate the tube and IMMUNOQUICK® MALARIA *falciparum* strip according to the procedure reserved to potentially infectious waste.

RESULTS

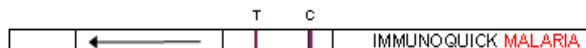
A proper reading requires:

- A minimum visual acuity
- Correct lighting conditions

POSITIVE :

Presence of 2 distinct bands:

- A purple control bands appears at the level of the control zone C, and
- A purple test band appears at the level of the test zone T



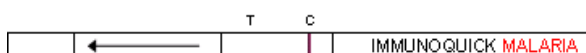
The result is positive even if the intensity of the purple test band T is very weak. The colored test band T may appear before the 10th minute in case of strong positive samples.

Note: In case of strong positive sample, the intensity of control line C may be weak

NEGATIVE:

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

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



INVALID:

Absence of purple control band C.

The procedure was not followed correctly or the test was deteriorated. Do not interpret the result; the test has to be performed with a new strip and a new plastic tube.



QUALITY CONTROL

- Internal procedural controls are included in the test. A coloured 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

1. IMMUNOQUICK® MALARIA *falciparum* cannot replace the microscopic examination which is recommended to use as a complement.
2. The clinical diagnosis has to be released by the physician on the basis of the other clinical and biological findings.
3. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
4. The strips and buffer of different lots must not be mixed and used.
5. In *P. falciparum*, HRP-2 is not secreted in gametogony stage. Hence, in "healthy carriers", the test band may be absent.
6. If the test is used as a control of therapy, it is recommended to carry out control at least fifteen days after the end of the treatment.

PERFORMANCES

1) Clinical evaluation

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:

Number of samples	Results of thin and/or thick films	IMMUNOQUICK® MALARIA <i>falciparum</i>	
		Negative	Positive
50	<i>Plasmodium falciparum</i> positive	0	50
15	Other <i>Plasmodium</i> positive	15	0
28	Absence of <i>Plasmodium</i>	27	1*

* This sample was negative by microscopic examination but was found positive on *Plasmodium falciparum* DNA PCR amplification assay confirming the high specificity of the test.

Sensitivity	Specificity	Negative predictive value	Positive predictive value
50 / (50 + 0)	42 / (42 + 1)	42 / (42 + 0)	50 / (50 + 1)
100 %	97.7 %	100 %	97.5 %
Confidence interval		Confidence interval	
92.9-100%		87.7-99.9%	

2) Interferences

The WHO evaluation report showed a false positive rate of 0.6% in 84 negative samples. No interference with other infectious diseases (Chagas, Dengue, Leishmania) was observed. Out of 54 samples positive with other immunological factors (rheumatoid factors, anti-nuclear antibodies, heterophile antibodies), the false positive rate has averaged 1.85%.

BIBLIOGRAPHY

1. 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.
2. Rock, E.P. et al 1987: Comparative Analysis of the plasmodium *falciparum* Histidine-Rich Proteins HPR-I HPR-II and HPR-III in Malaria Parasites of Diverse Origin. Parasitol, 95,209-227.
3. 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.
4. 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 *in vitro* diagnostic use only



Manufacturer



Store between 2-30°C



Do not reuse



Tests per kit



Catalog number



Expiry

Version 01 BR 06/2013



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