

Rapid Diagnostic kit for detecting infection with
P. falciparum Malaria Parasite in Human Whole Blood

INTRODUCTION

Malaria is a serious, sometimes fatal, parasitic disease characterized by fever, chills, and anaemia and is caused by a parasite that is transmitted from one human to another by the bite of infected Anopheles mosquitoes. There are four kinds of malaria parasite that can infect human: Plasmodium falciparum, P. vivax, P. ovale, and P. malariae. As per WHO estimation, in 2012, there were approximately 207 million cases and an estimated 627 000 deaths. Approximately 90% of all malaria deaths occur in sub-Saharan Africa, and 77% occur in children under 5 years. Malaria remains endemic in 104 countries, and, while parasite-based diagnosis is increasing, most suspected cases of malaria are still not properly confirmed, resulting in over-use of antimalarial drugs and poor disease monitoring. The use of antigen detecting rapid diagnostic tests (RDTs) is a vital part of malaria case management forming the basis for extending access to malaria diagnosis.

INTENDED USE

Advantage Pf Malaria Card is a visual, rapid qualitative and sensitive solid phase immuno chromatographic assay based on antigen detection and is as an aid in diagnosis of infection with HRP-2 (Histidine Rich Protein-2) specific P. falciparum in human whole blood specimens. The kit is intended for professional use and as a screening test and not to be used for carriers. All reactive samples should be confirmed by a supplemental assay like microscopic examination of thick smear and thin blood films.

It assists trained competent users in detecting plasmodium infections.

PRINCIPLE (ANTIGEN-ANTIBODY REACTION)

ADVANTAGE Pf MALARIA CARD is an immunoassay based on the "sandwich" principle. Colloidal gold is conjugated to monoclonal anti-Pf. specific HRP-2 antibody. The test uses monoclonal anti-Pf. HRP-2 antibody (test line Pf.) immobilized on a nitrocellulose strip. The test sample is added to the device. On addition of assay buffer, the red blood cells get lysed. If the sample contains P. falciparum, the colloidal gold antibody conjugate complexes the Pf. specific HRP-2 in the lysed sample. This complex migrates through the nitrocellulose strip by capillary action. When the complex meets the line of the corresponding immobilized monoclonal antibody, the complex is trapped forming a pinkish purple band which confirms a reactive test result. Absence of a coloured band in the test region indicates a non-reactive test result. A red procedural control line should always develop at 'C' region to indicate that the test has been performed properly.

MATERIALS PROVIDED

ADVANTAGE Pf MALARIA CARD Test kit contains following components to perform the assay:

S. No.	Component	25 Test Pack (Cat No.: IRC16025)
1.	Test Card	25 Nos.
2.	Assay Buffer	1 No. x 25 Tests
3.	Inverted Cup	1 Pack of 25 Nos.
4.	Instruction Manual	1 No.
5.	Swab	25 Nos.
6.	Sterile Lancet	25 Nos.

Material required, but not provided: Stop Watch.

KIT PRESENTATION

25 Test Pack

STORAGE AND STABILITY

ADVANTAGE Pf MALARIA CARD should be stored at 4-30°C in the coolest & driest area available. The kit has a shelf life of 24 months from the date of manufacturing. The kit should not be frozen & must be protected from exposure to humidity.

DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packaging. They are explained in more detail in the British and European Standard EN ISO 15223-1:2016.

	Manufactured By		In-vitro diagnostic medical device
	No. of tests		Consult Instructions for use
	Lot Number Batch Number		Temperature Limit
	Manufacturing Date		Caution
	Expiry Date		Catalogue Number
	Do not use if package is damaged		Authorized Representative in the European Community
	Single use only		Keep away from sunlight

WARNING FOR USERS

CAUTION: ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

- The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
- In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
- Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
- Tests are for in vitro diagnostic use only and should be run by competent person only.

- Do not pipette by mouth.
- All materials used in the assay and samples should be decontaminated in suitable disinfectant solution for 30-60 min. before disposal. They should be disposed off in accordance with established biosafety guidelines for handling & disposal of potentially infective material.
- Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
- Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
- Assay Buffer contains Sodium Azide (0.2%) as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds.
- Do not use the kit beyond the expiry date.
- Do not mix reagents from different batches.
- Do not open the foil pouch until it attains room temperature.
- Do not re-use the test device.
- Do not use any other buffer than the assay buffer supplied with this kit.
- Use separate Inverted Cup or pipette tips for each sample in order to avoid cross-contamination of samples which could cause erroneous results.
- Follow the given test procedure and storage instructions strictly.
- Dispose off the used lancets in sharps box.

SAMPLE / SPECIMEN COLLECTION AND STORAGE

- Collect the whole blood in a clean container (containing EDTA, citrate or heparin) by venipuncture. Fresh samples are preferred for testing as they perform best when tested immediately after collection. If samples are not immediately tested, they should be stored at 2-8°C for not more than 3 days, otherwise false / erroneous results may be obtained.
- Fresh blood from finger prick may also be used as a test sample.
- Heamolysed, lypaemic, ictric, clotted sample or sample with microbial contamination should not be used as can lead to erratic results.

BEFORE YOU START

- Open the kit and check for following kit components:



Test Device foil pouched with a desiccant.
Lot No., Mfg. & Exp. date are printed on back of the pouch.

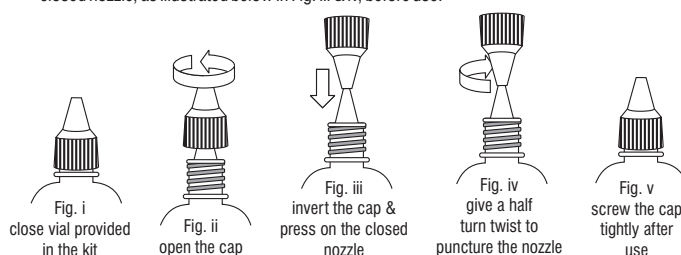


Assay Buffer



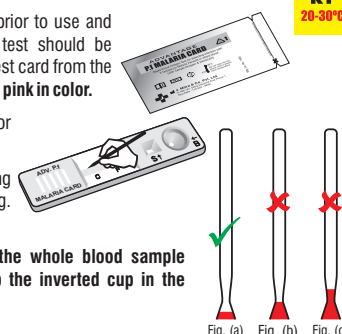
Inverted Cup

- The Assay Buffer Solution provided in the kit has closed nozzle and screw cap with pin (outside). Before using Assay Buffer, keep the vial vertically straight and tap down gently on the working platform, so that Assay Buffer comes down at the bottom of the vial and orifice the closed nozzle, as illustrated below in Fig. iii & iv, before use:



TEST PROCEDURE

- Bring the complete kit and specimen to be tested to room temperature prior to testing.
- Remove the test card from the foil pouch prior to use and place it on a flat and dry surface. The test should be performed immediately after removing the test card from the foil pouch. **Do not use the card if desiccant is pink in color.**
- Label the test card with patient's name or identification number.
- Take 4µl anti-coagulated whole blood using the Inverted Cup upto the mark as shown in fig. (a, b & c).



Note: Touch Inverted Cup to the surface of the whole blood sample collected in the container or tube. Do not dip the inverted cup in the container containing sample.

Fig. (a) Fig. (b) Fig. (c)

OR

Use finger prick blood sample as described below.

FINGER PRICK SAMPLE COLLECTION:

- Wipe the complete finger tip with the alcohol swab as shown in Fig. (D). Wait until the finger has completely dried (minimum 30 seconds).
 - Take the lancet and prick the side of the pulp (ball of the finger) with the lancet, perpendicular to the lines of the finger print as shown in Fig. (E).
 - Make sure a well formed drop of blood is present on the tip of the finger.
 - Take the Inverted Cup and collect 4µl of blood by touching the base of the Inverted Cup into the blood drop as shown in Fig. (f) and immediately place the Inverted Cup in the sample well "S". (Care should be taken that the blood sample does not clot & the transfer to the sample pad is immediate).
 - Add whole blood sample using the Inverted Cup/ micropipette onto the sample pad in the sample well 'S'. fig. (g)
- NOTE :** Press the base of the inverted cup onto the sample pad in the sample well "S" to ensure that the complete volume of whole blood has been transferred to the strip.
- Add 3 drops of the Assay Buffer in the buffer well 'B'. fig. (h). **Ensure FREE FALLING OF DROPS on the membrane, holding the vial/dropper vertically for proper volume. Screw cap the vial after use.**
 - Allow the reaction to occur for 20 minutes.
 - Read the results at 20 minutes. **Do not read the result after 20 minutes. Reading beyond prescribed time may give false results.**
 - Discard the Advantage Pf Malaria Card immediately after reading results at 20 minutes as it is potentially infectious.

INTERPRETATION OF THE RESULTS

REACTIVE

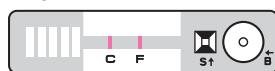


Fig. (i)

As shown in Fig. (i), appearance of two pinkish purple coloured line one each in Test region 'F' & Control region 'C' indicates that the sample is reactive for *P. falciparum*. A difference of intensity in colour may occur between the test line & control line depending on the concentration of HRP-2 antigen in the sample but this does not affect the interpretation of the results.

Depending on the concentration of HRP-2, positive results may be observed within 60 seconds. However, to confirm a negative result the test result should be read only at 20 minutes. If the conc. of HRP-2 in the sample is very high, only test line may be observed. Consider a faint test line also as positive result. This is due to Hook's effect. Such samples should be diluted 1:10 or 1:20 in negative blood (Human) & again re-run the test, Diluted sample should show both control & test line. In case, if control line does not appear or is faint dilute the sample further.

NON-REACTIVE



Fig. (j)

As shown in Fig. (j), appearance of only one pinkish purple coloured line at Control(C) region indicates that the sample is non-reactive for *P. falciparum*.

INVALID



Fig. (k)



Fig. (l)

The test is invalid, if no control line appears after the completion of test, either with clear background or with complete pinkish/ purplish background Fig. (k) & (l). **The test should be repeated using a new card.**

LIMITATIONS AND INTERFERENCES

- The test procedure, precautions and interpretation of results for this test must be strictly followed.
- As with all diagnostic tests, the test result must always be correlated with clinical finding.
- Though the test is accurate in detecting HRP-2 specific to *P. falciparum* or pLDH specific to Plasmodium species (*P. falciparum*/ *P. vivax*/ *P. malariae*/ *P. Ovale*), a low incidence of false results can occur. All reactive test results 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).

- This is only a screening test. The results of the 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.
- Since the HRP-2 persists for upto a fortnight even after successful therapy, a positive test result does not indicate a failed therapeutic response.
- In *P. falciparum* malaria infection, HRP-2 is not secreted in gametogony stage. Hence, in 'carriers', the HRP-2 test line (F) may be absent.
- The possibility of resistant strain of malaria should always be considered if the reaction of the test remains positive with the same intensity after 5-10 days post treatment.
- Patient with rheumatoid factors, anti-nuclear antibody or dengue may give false positive results.

PERFORMANCE CHARACTERISTICS OF ADVANTAGE Pf MALARIA CARD

(i) WHO Evaluation:

The ADVANTAGE Pf MALARIA CARD test kit has been evaluated by WHO, Geneva using a panel of wild & cultured malaria positive sample and the results obtained are as follows :

	Panel Detection Score	Specificity
200 Parasites	89%*	—
2000 Parasites	99%*	—
Negative Samples	—	100%*

*Reference: Malaria Rapid Diagnostic Test Performance: Results of WHO product testing of malaria RDTs: Round 5 (2013), Page:38.

Note : The above information is provided for the scientific community, It is not for commercial or promotional purpose.

(ii) In-house Evaluation:

The test can detect parasitemia levels of ≥ 100 parasites per μl of blood for *P.f* (HRP-2). The Advantage Pf Malaria Card kit has been evaluated in-house with malaria positive and negative clinical whole blood samples and compared with microscopic examination. The evaluation also included cross-reacting samples; Dengue, Rheumatoid factor, HIV, HCV, HBV, ASO and Syphilis positive samples. The results obtained are as follows:

Sample	Total no. of samples tested	ADV. Pf MALARIA CARD		Sensitivity (%)	Specificity (%)
		Positive	Negative		
Malaria Negative	700	0	700	-	100
Cross-reacting sample	14	0	14	-	100
<i>P. falciparum</i> Positive	57	57	0	100	-
<i>P. vivax</i> Positive	3	0	3	-	100

Precision: Within-run and between-run precisions have been determined by testing 10 replicates of 6 specimens : 4 negative and 6 positive samples (1 strong, 1 medium and 4 weak). The CV% of negative and all positive samples were within 10% of the time.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application thereof.

BIBLIOGRAPHY OF SUGGESTED READING

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For in-vitro diagnostic use only, not for medicinal use

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