ADVANTAGE

MALARIA CARD

Rapid Antigen Test for detecting infection with P. falciparum and P. vivax 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 parasite that can infect human : Plasmodium falciparum, P. vivax, P. ovale, and P. malariae. The disease now occurs in more than 90 countries worldwide, and it is estimated that there are over 500 million clinical cases and 2.7 million malaria-caused deaths per year. At present, malaria is diagnosed microscopically by looking for the parasites in a drop of blood.

INTENDED USE

ADVANTAGE MALARIA CARD is a visual, rapid and sensitive solid phase immunochromographic assay for the qualitative differential detection of P. falciparum (HRP-2) and P. vivax malaria antigen in Human Whole Blood only. The kit is intended for professional use and as a screening test. All reactive samples should be confirmed by a supplental 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 MALARIA CARD is an immunoassay based on the "sandwich" principle. Colloidal gold is conjugated to P.f specific monoclonal anti-HRP-2 antibody and monoclonal anti-pan specific pLDH antibody. The test uses monoclonal anti-P.f. HRP-2 antibody (test line F) & monoclonal anti-P.v. specific pLDH antibody (test line V) 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 or P. vivax or both, the colloidal gold conjguate complexes the HRP-2 / P. vivax specific pLDH in the lysed sample. This complex migrates through the nitrocellulose strip by capillary action. When the complex meets the line of the corresponding immobilized antibody, the complex is trapped forming a pink 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 MALARIA CARD Test kit contains following components to perform the assay:

S. No.	Component	50 Test Pack Cat No.: IR211050
1.	Advantage Malaria Card	50 nos.
2.	Assay Buffer	2 nos. x 25 Tests
3.	Sample Dropper	1 pack of 50 nos.
4.	Instruction for use	1 No.
5.	Swab & Lancet (Available on request)	50 Nos. each

KIT PRESENTATION

50 Test Pack

ADDITIONAL MATERIALS REQUIRED, BUT NOT PROVIDED

- Timer or Stop Watch

STORAGE AND STABILITY

ADVANTAGE MALARIA CARD should be stored at 4-30ºC in the cool & driest area available. Expiry date on the kit indicates the date beyond which the kit should not be used. 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 packing. They are explained in more detail in the European Standard EN ISO 15223-1:2021.

	Manufactured By	IVD	In vitro diagnostic medical device
Σ	No. of tests		Instruction for use
LOT	Lot Number Batch Number	4°C 30°C	Temperature Limitation
	Manufacturing Date		Caution, see instruction for use
	Expiry Date	REF	Catalogue Number
	Do not use if package is damaged	BIO	Contains biological Material of Animal Origin
2	Single use only		Keep away from sunlight
	Keep Dry	IN	Country of Manufacture

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.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7. All materials used in the assay and samples should be decontaminated in suitable disinfectant solution for 30-60 min. before disposal or by autoclaving at 121ºC at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established biosafety guidelines for handling & disposal of potentially infective material.
8. 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.
9. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
10. Assay Buffer contains Sodium Azide 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. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976).
11. Do not use the kit beyond the expiry date.
12. Do not mix reagents from different batches.
13. Do not open the foil pouch until it attains room temperature.
14. Do not re-use the test device.
15. Do not use any other buffer than the assay buffer supplied with this kit.
16. Use separate sample dropper or pipette tips for each sample in order to avoid cross-contamination of samples which could cause erroneous results.
17. Follow the given test procedure and storage instructions strictly.
18. Dispose off the used lancets in sharps box.

SAMPLE / SPECIMEN COLLECTION AND STORAGE

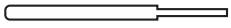
1. Collect the whole blood in a clean container (containing EDTA, citrate or heparin) by venipuncture. Fresh samples are preferred for testing as they perform best when tested immediately after collection. If samples are not immediately tested, they should be stored at 2-8ºC for not more than 3 days,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

BEFORE YOU START

1. Open the kit and check for following kit components:

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

Assay Buffer



Sample Dropper

2. 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:

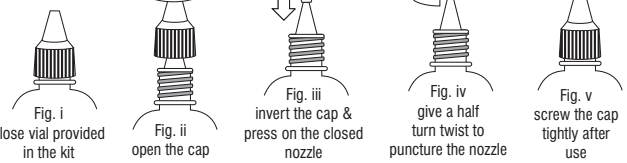


Fig. i close vial provided in the kit

Fig. ii open the cap

Fig. iii invert the cap & press on the closed nozzle

Fig. iv give a half turn twist to puncture the nozzle

Fig. v screw the cap tightly after use

TEST PROCEDURE

RT 20-30ºC

1. Bring the complete kit and specimen to be tested to room temperature minimum 30 minutes prior to testing.
2. 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.

Note: Do not use the card if desicant is pink in color.

3. Write the patient's name or identification number on the test card.
4. Take 4µl anti-coagulated whole blood using the sample dropper upto the mark as shown in fig. (b).

Note: Sample taken above the mark as shown in fig. (a) and sample taken below the mark as shown in fig. (c) are wrong and will lead to erratic results.

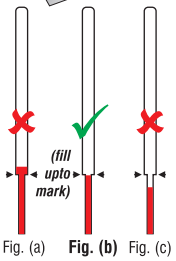


Fig. (a) **Fig. (b)** Fig. (c)

OR

Use finger prick blood sample as described below.

FINGER PRICK SAMPLE COLLECTION:

a Wipe the complete finger tip with the alcohol swab. Wait until the finger has completely dried (minimum 30 seconds).

b 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.

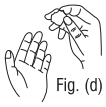


Fig. (d)

c Make sure a well formed drop of blood is present on the tip of the finger.

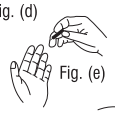


Fig. (e)

d Take the sample dropper and collect 4μl of blood by dipping the tip of the sample dropper into the blood drop as showin in fig. (g) and immediately place the tip of the sample dropper in the sample well "S". Press the tip of the dropper onto the sample pad in the sample well "S" to ensure that the complete volume of whole blood has been transferred to the strip. (Care should be taken that the blood sample does not clot & the transfer to the sample pad is immediate).


Fig. (f)

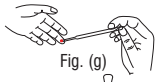


Fig. (g)

OR

Alternatively, using a micropipette take 4μl of the finger prick specimen.

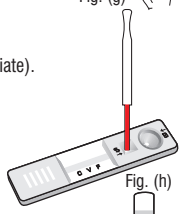


Fig. (h)

5 Add whole blood sample using the sample dropper/ micropipette onto the sample pad in the sample well 'S'. fig. (h)

NOTE : Make sure that the blood from the sample dropper has been completely transferred to the sample pad.

6. Add 3 drops of the Assay Buffer in the buffer well 'B'. fig. (i). **Ensure FREE FALLING OF DROPS on the membrane, holding the vial/dropper vertically for proper volume. Screw cap the vial after use.**

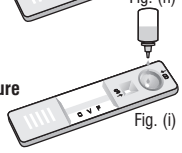


Fig. (i)

7. Allow the reaction to occur for 20 minutes.
8. Read the results at 20 minutes. **Do not read the result after 20 minutes. Reading beyond prescribed time may give false results.**
9. Discard the ADVANTAGE MALARIA CARD immediately after reading results at 20 minutes as it is potentially infectious.

INTERPRETATION OF THE RESULTS

REACTIVE

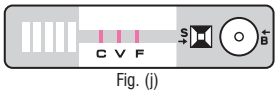


Fig. (j)

As shown in fig. (j), appearance of three pink coloured line one each in P.v. region (V), P.f. region (F) & Control region (C) indicates that the sample is reactive for P. falciparum and P. vivax.

Fig. (k)

As shown in fig. (k) appearance of two pink coloured line one each at V & C region only indicates that the sample is reactive for P. vivax only.

Fig. (l)

As shown in fig. (l) appearance of two pink coloured line one each at F & C region only indicates that the sample is reactive for P. falciparum only. A difference of intensity in colour may occur between the test line & control line depending on the concentration of HRP-2 / pLDH in the sample but this does not affect the interpretation of the results.

Depending on the concentration of pLDH/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 pLDH/HRP-2 in the sample is very high, only test line may be observed. 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. **Consider a faint test line also as a positive result.**

NON-REACTIVE

Fig. (m)

As shown in fig. (m), appearance of only one pink coloured line at Control(C) region indicates that the sample is non-reactive for P. vivax and P. falciparum.

INVALID

Fig. (n)

Fig. (o)

Fig. (p)

Fig. (q)

The test is invalid, if no line appears after the completion of test, either with clear background or with complete pinkish/ purplish background fig. (n, o, p & q). **The test should be repeated using a new card.**

LIMITATIONS AND INTERFERENCES

1. The test procedure, precautions and interpretation of results for this test must be followed strictly.
2. As with all diagnostic tests, the test result must always be correlated with clinical finding.
3. **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).
4. Any modification to the above procedure and / or use of other regaents will given erractic results.
5. In P. falciparum malaria infection, HRP-2 is not secreted in gametogony stage. Hence, in "Carriers", the HRP-2 band may be absent.
6. Since the HRP-2 level persists for upto 15 days even after successful therapy, a reactive test result does not indicate a failed therapeutic response.
7. Patient with rheumatoid factors, anti-nuclear antibody or dengue may give false positive results.

PERFORMANCE CHARACTERISTICS OF ADVANTAGE MALARIA CARD

In-house Evaluation:

Analytical Sensitivity: The test can detect parasitemia levels of >100 parasites per μl of blood for P. falciparum (HRP-2) and >200 parasites per μl of blood for P. vivax (pLDH) .

The ADVANTAGE MALARIA CARD 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, Leptospira, HIV, HCV, HBV, M. tuberculosis, Syphilis, Brucella, Scrub typhus positive samples. The results obtained are as follows:

Sample	Total no. of samples tested	ADV. MALARIA CARD		Sensitivity (%)	Specificity (%)
		Positive	Negative		
Malaria Negative	2010	1	2009	-	99.95
Cross-reacting sample	59	0	59	-	100
P. falciparum Positive	58	58	0	100	-
P. vivax Positive	105	105	0	-	100

External Evaluation:

The Advantage Malaria Card test kit has been evaluated by ICMR- National Institute of Malaria (NIMR), New Delhi, using panel of malaria positive (P.f & P.v) and negative samples and the results obtained are as follows:

Sample	Panel detection Score	Specificity
P. falciparum (200 Parasite /μl)	100%	-
P. vivax (200 Parasite /μl)	100%	-
Negative Sample	-	100%

Precision: Within-run and between-run precisions have been determined by testing 10 replicates of seven specimens : one negative, three P.f (weak positive) and three P.v (one weak and two medium) positive samples. The C.V (%) of negative, weak positive and strong 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 for use, 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 there of.

BIBLIOGRAPHY OF SUGGESTED READING

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in vitro diagnostic reagent, not for medicinal use

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