ADVANTAGE

MAL CARD

Rapid Diagnostic Antigen test kit for detecting infection with P. falciparum (pLDH) and Plasmodium Species (pLDH)(P. falciparum / P. vivax / P. malariae / P.ovale) 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 an 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 MAL CARD is a visual, rapid, qualitative and sensitive solid phase immuno chromatographic assay based on antigen detection and is as an aid in the qualitative diagnosis of infection with pLDH (Plasmodium Lactate Dehydrogenase) antigen specific to Plasmodium falciparum and other Plasmodium Species (P. vivax / P. malariae / P. ovale) in human whole blood. 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 MAL CARD is an immunoassay based on the "sandwich" principle. The conjugate contains colloidal gold conjugated to monoclonal anti-pan specific pLDH (Plasmodium Lactate Dehydrogenase) antibody. The test uses monoclonal anti-Pf pLDH antibody (test line F) & monoclonal anti-Pan specific pLDH antibody (test line P) 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 and/or P. vivax/P. malariae/P. ovale, the colloidal gold antibody conjugate complexes the P.f. specific pLDH/Pan 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 monoclonal antibody, the complex is trapped forming a purplish pink 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.

KIT PRESENTATION & MATERIALS PROVIDED

ADVANTAGE MAL CARD Test kit contains following components:

S. No.	Component	50 Test Pack Cat No.: IR221050	100 Test Pack Cat No.: IR221100
1.	Advantage Mal Card	50 nos.	100 nos.
2.	Assay Buffer	2 nos. x 25 Tests	4 nos. x 25 Tests
3.	Sample Dropper	1 pack of 50 nos.	2 packs of 50 nos.
4.	Instruction Manual	1 No.	1 No.

Optional material required, but not provided: Calibrated micropipette capable of delivering 4μl sample, swab and Sterile Lancet.

KIT PRESENTATION

50 Test Pack 100 Test Pack

STORAGE AND STABILITY

ADVANTAGE MAL 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 ISO15223-1:2021.

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

WARNING & PRECAUTIONS 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.

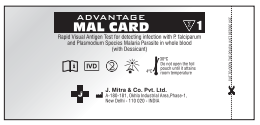
1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. 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.
7. 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.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. 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.
10. Do not use the kit beyond the expiry date.
11. Do not mix reagents from different batches.
12. Do not open the foil pouch until it attains room temperature.
13. Do not re-use the test device.
14. Do not use any other buffer than the assay buffer supplied with this kit.
15. Use separate sample dropper or pipette tips for each sample in order to avoid cross-contamination of samples which could cause erroneous results.
16. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
17. Follow the given test procedure and storage instructions strictly.
18. Dispose off the used lancets in sharps box.
19. **The performance of Advantage Mal Card has not been evaluated with P. malarie and P. ovale positive samples.**

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.

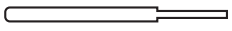
BEFORE YOU START

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

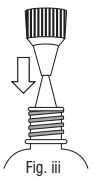


Fig. iii
invert the cap & press on the closed nozzle

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

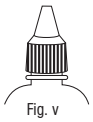
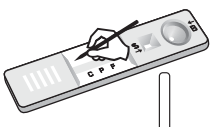


Fig. v
screw the cap tightly after use

TEST PROCEDURE

1. Bring the complete kit and specimen to be tested to room temperature prior to testing. **RT 20-30°C**
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. **Do not use the card if desicant is pink in color.**



3. Label the test card with patient's name or identification number.
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.

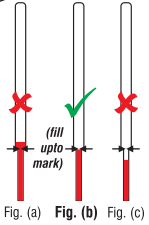


Fig. (a) **Fig. (b)** Fig. (c)

OR

Use finger prick blood sample as described below.

FINGER PRICK SAMPLE COLLECTION:

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

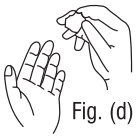


Fig. (d)

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 as shown in Fig. (e).

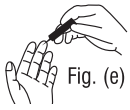


Fig. (e)

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


Fig. (f)

d Take the sample dropper and collect 4μl of blood by dipping the tip of the sample dropper into the blood drop as shown in Fig. (f) and Immediately place the tip of the sample dropper in the sample well "S". **(Care should be taken that the blood sample does not clot & the transfer to the sample pad is immediate)**.

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

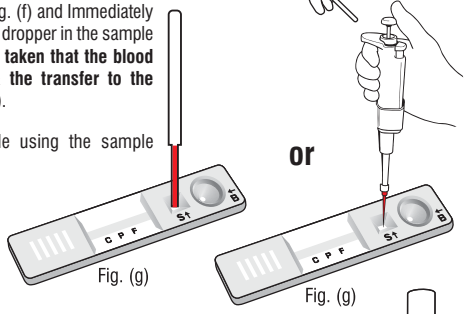


Fig. (g) Fig. (g)

NOTE :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.

6. 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.**

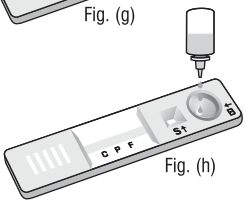


Fig. (h)

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 MAL 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 three purplish pink coloured lines one each in P.f. region (F), Pan region (P) & Control region (C) indicates that the sample is reactive for P. falciparum or mixed infection of P.f and P. vivax or (P. malariae / P. ovale).

Fig. (j)

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

A difference of intensity in colour may be observed between both the test lines ('P' & 'F') and between the test lines and control line from faint to strong intensity depending on the concentration of pLDH antigen in the sample. In case of faint test line ('P' and/or 'F') repeat the test using fresh card.

NON-REACTIVE

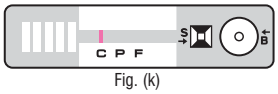


Fig. (k)

As shown in fig. (k), appearance of only one purplish pink coloured line at Control (C) region indicates that the sample is non-reactive for P. falciparum and other Plasmodium Species (P. vivax / P. malariae / P. ovale).

INVALID

Fig. (l)

Fig. (m)

Fig. (n)

Fig. (o)

The test is invalid, if or no control line appears after the completion of test either with clear background or with complete pinkish/ purplish background fig. (l, m, n & o). **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 strictly followed.
2. As with all diagnostic tests, the test result must always be correlated with clinical finding.
3. Though the test is accurate in detecting pLDH specific to P. falciparum and other Plasmodium species (P. vivax/ P. malarie/ 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).
4. Any modification to the above procedure and / or use of other reagents will lead to erretic results.
5. In case of faint test line, repeat the test using fresh card. If faint test line persist, confirm the test result with ELISA/Clia/ microscopy and co-relate clinically.
6. In most of the cases, after successful anti-malarial therapy the Pan band will turn negative. However, depending on the medication used, the clearance of parasite may take longer and the reaction of the test may remain positive. In such cases the test should be repeated after 5-10 days of start of treatment.
7. In case only "F" test line appears and "P" line is absent on the card for a sample, then confirm the result with microscopy . IF the sample is negative by microscopy then interpret the result as Malaria Negative . This could be due to cross reactivity with Rheumatoid factor and heterophilic antibodies.
8. 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.
9. Patient with rheumatoid factors, anti-nuclear antibody or dengue may give false positive results.

PERFORMANCE CHARACTERISTICS OF ADVANTAGE MAL CARD

(i) WHO Evaluation:

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

	Panel Detection Score*	Specificity*
200 Parasites	94.3% (P.v)	—
2000 Parasites	94.0% (P.f) and 97.1% (P.v)	—
Negative Samples	—	99.6%

*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:

Analytical Sensitivity: The test can detect parasitemia levels of $\geq$100 parasites per μl of blood for P. falciparum (pLDH) and $\geq$200 parasites per μl of blood for P. vivax (pLDH) .

The ADVANTAGE MAL 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. MAL 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

Precision: Within-run and between-run precisions have been determined by testing 10 replicates of 8 specimens: four negative and four positive; two weak and two moderate positive. The C.V (%) of negative, moderate positive and weak 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 there of.

BIBLIOGRAPHY OF SUGGESTED READING

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4. Moody A, Clinical Microbiological Review, 15(1), 2002, 66-78.

VER-03 R-08

in vitro diagnostic reagent, not for medicinal use

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MN/ACM/001
Rev. Date: Jul.-22