ADVANTAGE

P.f MALARIA CARD

Product Code: (IRC16025)

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[1]. There are four kinds of malaria parasite that can infect human: Plasmodium falciparum (P.f), 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[2]. 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 P.f Malaria Card is a visual, rapid and qualitative solid phase immuno-chromatographic assay based on HRP-2 (Histidine Rich Protein-2) specific P. falciparum antigen detection in human venous and capillary whole blood specimens[3]. The test kit is intended for professional use as well as an aid to the diagnosis of malaria infection.

The assay assists trained competent users in either laboratory or point of care settings and is to be used for diagnosis of malaria in symptomatic adult and paediatric (10 months and older) individuals suspected of malaria. The assay is performed manually and is not intended for self-testing.

PRINCIPLE

Advantage P.f Malaria Card is an immunoassay based on the "sandwich" principle. Colloidal gold is conjugated to monoclonal anti-P.f specific HRP-2 antibody. The test uses monoclonal anti-P.f HRP-2 antibody (test line P.f) 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 P.f 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 colored 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 CONTENTS

Advantage P.f Malaria Card Test kit contains following components to perform the assay:

S. No.	Component	25 Test Pack (Cat No.: IRC16025)
1.	Test Card (containing 1 test device & 1 desiccant)	25 Nos.
2.	Assay Buffer Bottle	1 No.(4 ml)
3.	Sample Transfer Device (Inverted Cup)	1 Pack of 25 Nos.
4.	Alcohol Swab	25 Nos.
5.	Sterile Lancet	25 Nos.
6.	Instruction for use	1 No.

Active Ingredients of main component:

- **1 Test Card includes:**
  - Detection antibodies conjugated to colloidal gold :
    - Mouse monoclonal antibodies specific to Pf-HRP-II-gold Colloid
    - Chicken IgY – gold Colloid
  - Capture antibodies:
    - Test line (F)- Mouse monoclonal antibodies specific to Pf-HRP-II
    - Control line(C): Anti-Chicken IgY polyclonal antibodies
- **Assay Buffer:** Protein stabilizers, Detergent and Preservatives

Material required, but not provided: Timer, Sharp box, pen, disposable gloves, non-sharp disposable container, micropipette and microtips in case of venipuncture blood collection, and extra lancets and alcohol swabs, if needed (lancet misfires, lancet does not produce sufficient blood volume, alcohol swab is dried out, etc.).

KIT PRESENTATION

25 Test Pack

STORAGE AND STABILITY

Advantage P.f Malaria Card should be stored at 4-30ºC in the coolest & driest area available. The kit has a shelf life of 30 months from the date of manufacturing. The kit should not be frozen & must be protected from exposure to humidity.

WARNING AND PRECAUTIONS

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 (0.2%) as a preservative. Sodium Azide may be toxic if ingested. If these materials 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. Do not use the test if the packaging is damaged.
16. Do not use any other specimen than whole blood.
17. Do not use if the product has been exposed to excessive heat or humidity.
18. Use separate Inverted Cup or pipette tips for each sample in order to avoid cross-contamination of samples which could cause erroneous results.
19. Follow the given test procedure and storage instructions strictly. Failure to follow the test procedure and instructions strictly can cause improper functioning of the test and inaccurate results.
20. Dispose off the used lancets in sharps box.
21. Dispose off the inverted cup, test device, swab and microtips in non-sharp disposal bag/ container.

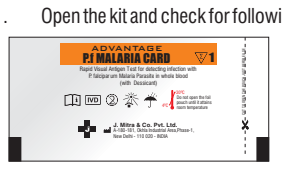
SAMPLE / SPECIMEN COLLECTION AND STORAGE

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. Hemolyzed, lipemic, icteric, clotted sample or sample with microbial contamination should not be used as can lead to erratic results.

BEFORE YOU START

READ THE INSTRUCTIONS CAREFULLY BEFORE PERFORMING THE TEST

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



Individually foil pouched Test Card (containing 1 test device & 1 desiccant) Lot No., Mfg. & Exp. date are printed on back of the pouch.



Assay Buffer Bottle

Sample Transfer Device (Inverted Cup)



Alcohol Swab



Sterile Lancet



Instruction for use

2. Check the test device pouch for proper sealing and/or any damage. Discard the test device if damaged and use another test device.
3. Check the color of the desiccant and it should be blue.
4. The Assay Buffer Bottle provided in the kit has closed nozzle and screw cap with pin (outside) (fig-i). 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 (fig-ii). Orifice the closed nozzle (fig. iii & iv) before use. Screw the cap tightly on the bottle after use (fig-v) as illustrated below in fig. i to fig. v.



Fig. i close vial provided in the kit



Fig. ii open the cap

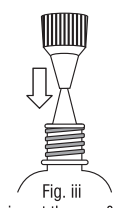


Fig. iii invert the cap & press on the closed nozzle

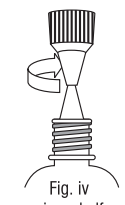


Fig. iv give a half turn twist to puncture the nozzle

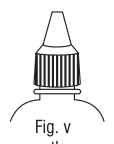


Fig. v screw the cap tightly after use

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 desiccant is pink in color.



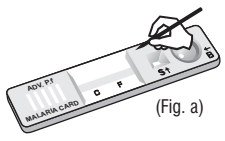
Dessicant in good condition



Pink color dessicant

Do not use the Card if dessicant is pink in color

3. Label the test card with patient's name or identification number as shown in (fig. a).



(Fig. a)

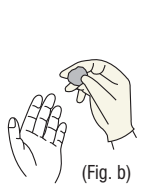
Capillary Whole Blood collection from finger prick:

a Wipe the complete fingertip with the alcohol swab as shown in (fig. b). 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 as shown in (fig. c).

c Make sure a well formed drop of blood is present on the tip of the finger as shown in (fig. d).

4. Take the Inverted Cup and collect 4μl of capillary blood by touching the base of the Inverted Cup into the blood drop as shown in (fig. e) and Immediately place the sample transfer device (Inverted Cup) in the sample well "S"as shown in (fig. f). (Care should be taken that the blood sample does not clot & the transfer to the sample pad is immediate).



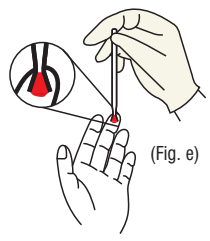
(Fig. b)



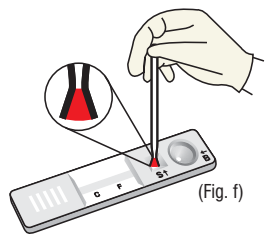
(Fig. c)



(Fig. d)



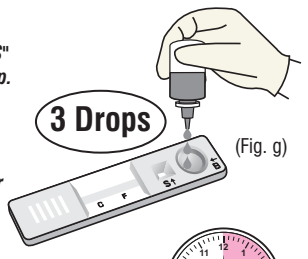
(Fig. e)



(Fig. f)

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.

5. Add 3 drops of the Assay Buffer in the buffer well 'B'. as shown in (fig. g).



(Fig. g)

Note: Ensure FREE FALLING OF DROPS on the buffer well, holding the vial/dropper vertically for proper volume. Screw cap the vial after use.

6. Allow the reaction to occur for 20 minutes.



20 minutes

7. Read the results at 20 minutes. Do not read the result before 20 minutes or after 30 minutes of adding Assay Buffer. Reading before or beyond prescribed time may give false results.
8. Discard the Advantage P.f Malaria Card immediately after reading results at 20 minutes considering it to be potentially infectious.

Venous Whole Blood from venipuncture:

a Wear gloves.

b Collect whole blood by standard vanipuncture procedure into a tube containing anti-coagulant (EDTA, heparin or citrate).

c Mix the content of the sample tube gently before use.

d. Take 4μl of anti-coagulated whole blood using sample transfer device (inverted cup) and transfer the sample in the sample well "S" of the test card. Alternatively take 4 μl whole blood using micropipette.

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.

e. Peform steps 5 to 8 of the previous section of "Test Procedure"

INTERPRETATION OF TEST RESULTS

Lines that you Observe after running the test	Appearance of lines in device result window	Result interpretation
2 Pinkish purple line : at "C", "F"	C F	Positive for P. falciparum
1 Pinkish purple line at "C"	C F	Negative for P. falciparum
No Pinkish purple line at "C"	C F C F	Invalid result. Repeat the test using new device.

Note: A difference of intensity in color may occur between the test line ('F') and control line ('C') depending on the concentration of HRP-2 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. Consider a faint test line also as a positive result. if repeated invalid results or an increased rate of invalid test results are obtained, the end user should contact us at +91-11-47130300,500 or write us at jmitra@jmitra.co.in and the local regulatory authority.

LIMITATIONS OF THE TEST

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 findings.
3. Though the test is accurate in detecting HRP-2 specific to P. falciparum, a low incidence of false results can occur[4]. 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 invalidate the test procedure.

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5. Since the HRP-2 persists for upto a fortnight even after successful therapy, a positive test result does not indicate a failed therapeutic response[5].
6. 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[6].
7. 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[7].
8. Presence of heterophile antibodies in patient's sample other than Rheumatic diseases and autoimmune disorder may lead to false results. Interfering substances, cross reacting factors other than those tested in Analytical specificity may affect the performance of the kit[8,9&10].
9. The control line (C) only indicates the flow of diluent but does not specifically indicate that specimen has been successfully applied to the test.
10. The product performance will be hampered or degraded if test kit is stored at low temperature (<2ºC) or high temperature (>30ºC).
11. False negative results may be obtained in following conditions:

    i) Parasite density/ antigen concentration is below the detection limit of the test or analyte detected are not present during the stage of disease in which specimen is collected.

    ii) No production of HRP-2 antigen in the specimen due to deletion of HRP-2 gene[7].
12. A high fraction of interstitial fluid due to "milking" of the fingertip may be a cause of false negative results
13. Absence of hook-effect has been observed with parasite density up to 9900 parasites/μl.
14. In case of very faint or doubt for test band (F), the test should be repeated using fresh device.
15. Repeat the test in case of strong clinical evidence of malaria using fresh device.

PERFORMANCE CHARACTERISTICS OF ADVANTAGE P.f MALARIA CARD

I) Clinical performance:

Diagnostic Sensitivity and specificity:

a) With Venous Whole Blood samples:

i) **In-House Evaluation:** The ADVANTAGE P.F MALARIA CARD has been evaluated with malaria positive and negative clinical whole blood samples (venous whole blood), including cultured derived parasite sample and compared with microscopic examination. The results obtained are as follows:

Sample	Total no. of samples tested	Diagnostic Sensitivity (%)	Diagnostic Specificity (%)	Senstivity at 95% CI	Specificity at 95% CI
Malaria Negative	700	-	100	-	99 to 100%
P. falciparum Positive	25	100	-	86.28 to 100%	-

ii) **External Evaluation:** The ADVANTAGE P.F MALARIA CARD has been evaluated with malaria positive and negative clinical whole blood samples (venous whole blood), at 3 different sites; JMD Diagnostics Pvt. Ltd., Kolkata, India (47 P.f Positive and 51 Negative (22 Malaria Negative and 31 P.v Positive)) SDACC Healthcare Pvt. Ltd., Bangalore, India (72 P.f Positive and 50 Negative) and Yenepoya Medical Collage Hospital, Mangalore (27 P.f Positive and 63 Negative (35 Malaria Negative and 28 P.v Positive))and compared with microscopic examination. The results obtained are as follows:

Sample	Total no. of samples tested	Diagnostic Sensitivity (%)	Diagnostic Specificity (%)	Senstivity at 95% CI	Specificity at 95% CI
Malaria Negative	166	-	100	-	97.1 to 100%
P. falciparum Positive	146	100	-	96.8 to 100%	-

b) Capillary Blood samples: The diagnostic sensitivity and specificity has been evaluated using capillary samples from symptomatic patients in comparison to microscopy at 4 sites - People's Polyclinic,Nellore ,A.P., INDIA (12 P.f Positive and 325 Negative), District Vector Borne Disease Control office, Mangalore, Karnataka, INDIA (7 P.f Positive and 141 negative), SDACC Healthcare Pvt. Ltd., Bangalore, India (35 P.f Positive and 30 Negative) & Universite De Lomo ,Togo (225 P.f Positive and 164 negative). The results obtained are as follows:

Sample	Total no. of samples tested	Diagnostic Sensitivity (%)	Diagnostic Specificity (%)	Senstivity	Specificity
Malaria Negative	660	-	100	-	99.27 to 100%
P. falciparum Positive	279	100	-	98.30 to 100%	-

II) Analytical Performance:

a) Analytical Sensitivity: The sensitivity of the kit has been determined for HRP-2 Antigen using 1ST WHO international standard for P.falciparum antigen NIBSC Code No. 16/376 and it is equal to 62.5 IU/ml.

b) Analytical Specificity: The analytical specificity of Advantage P.f Malaria Card has been evaluated using potentially cross-reacting samples (208) ; Dengue, Leptospira, HIV, HCV, HBV, M.tuberculosis, Syphilis, Brucella, Scrub typhus , Hepatitis A , Measles ,Toxoplasma, Yellow fever, HAMA ,Influenza A virus and Leishmania positive samples.

The performance is also checked using potentially interfering substances; Low and elevated levels of bilirubin(upto 12 mg/dl), triglyceride(upto 964mg/dl), protein(upto 9g/dl), Albumin (upto 5 gm/dl), haemoglobin(upto 23.1 mg/dl), Rheumatoid factor(upto 79 IU/ml), Antenatal, ANA, Elevated IgM (upto 600mg/dl)& IgG (upto 3200mg/dl) and the effect of Relevant medicines; including anti-parasitic, antimalarial, antiretroviral and anti-tuberculosis medicines; Isoniazid (1.21 g/ml), Ethambutol (5.9 g/ml), Rifampicin (31.0 g/ml), Azithromycin (0.45 g/ml), Oseltamivir(10mg/ml), Doxycycline (70 M) Quinine (150 M), Erythromycin (81.6), Ciprofloxacin (30.2 M), and over-the-counter anti-inflammatory medications; Aspirin (44.5 g/ml), Aceptamenophen (199 M), Ibuprofen (2.425 mM), Caffein (2.0 g/ml) at their physically relevant dosage on test results of Advantage P.f Malaria Card. There is no interference on test results of Advantage P.f Malaria card kit with above mentioned cross reacting and potentially interfering substances at their physiologically relevant concentration /dosage.

c) Precision (Reproducibility & Repeatability): Within-run and between-run precisions have been determined by testing 10 replicates of eight samples; Two negative and 6 P.f positive; 1 (Strong), 2 (medium) and 3 (weak) positive samples in 3 different lots at manufacturer end . The reproducibility (inter-assay) was checked with above mentioned 8 samples in 10 replicates in 10 different runs in 3 days by the same analyst.

Repeatability (Intra Assay) testing of Advantage P.f Malaria Card is done by running 8 samples in 10 replicates in 1 run at a time by the same analyst.

The C.V (%) of negative & all positive samples were within 10% of the time in precision studies.

The Reproducibility testing has also been conducted at 1 customer end; AGM Diagnostic Centre and clinic, Kolkata with 6 P.f positive samples(2 strong and 4 weak positive) and 2 negative samples in 3 different runs by one analyst in a single day . No difference in test results were observed in Reproducibility studies conducted by lab.

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.

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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		See 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	IN	Country of Manufacture
2	Single use only		Keep away from sunlight
	Keep Dry	BIO	Contains biological Material of Animal Origin
	Acute Toxic		

in vitro diagnostic reagent, not for medicinal use

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