

WHO Prequalification of In Vitro Diagnostics PUBLIC REPORT

Product: AdvDx Malaria Pf Rapid Malaria Ag Detection Test WHO reference number: PQDx 0345-101-00

AdvDx Malaria Pf Rapid Malaria Ag Detection Test with product code 00-DKM-RK-MAL-ADX-004-025, manufactured by **Advy Chemical Pvt Ltd., rest-of-world regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 16 May 2019.

Summary of WHO prequalification assessment for AdvDx Malaria Pf Rapid Malaria Ag Detection Test

	Date	Outcome
Prequalification listing	16-05-2019	listed
Dossier assessment	05-12-2018	MR
Site inspection(s) of quality management system	02-10-2017	MR
Product performance evaluation	2016	MR

MR: Meets Requirements

Intended use

According to the manufacturer *“AdvDx Malaria Pf test kit is an in-vitro diagnostic Immunochromatographic assay for the qualitative detection of malaria infection caused by Plasmodium falciparum parasites in human. It detects HRP-II (Histidine Rich Protein-II) antigen of Plasmodium falciparum in whole blood specimens. It does not assess parasite densities. The test must be performed by trained professional user and not by lay users.”*.

Assay description

According to the manufacturer *“AdvDx Malaria Pf test utilizes the principle of Immuno-chromatography. It has the test strip coated with Monoclonal Anti-HRP-II (Test line Pf) which is specific to the histidine rich protein II of P. falciparum. As the test sample flows through the membrane assembly of the device after addition of the buffer solution, the colored colloidal gold, and the anti- HRP-II antibody conjugate complexes with the lysed blood sample. The malarial antigens get immobilized on the respective test lines on the nitrocellulose membrane which leads to the formation of red/purple colored band/s. The unreacted conjugate continues to migrate and is subsequently immobilized at the control “C” region forming a red/purple band. The control band demonstrates that liquid has migrated, but does not demonstrate that the assay procedure has been followed correctly.*

The control band must appear to prove that sample or buffer have migrated though it does not indicate that the specimen is correct and the assay procedure is followed correctly”.

Test kit contents

Component	25 tests/kit (00-DKM-RK-MAL- ADX-004-025)
AdvDx Malaria Pf Test Device individually foil pouched with a Desiccant	25
Sample applicator	
Alcohol Swab	
Lancet	
Buffer solution	1
Product Insert (IFU)	

Items required but not provided

- Timer;
- New pair of disposable gloves;
- Pen/pencil;
- Biosafety sharps container;
- Biohazard waste container (for potentially infectious waste);
- If whole blood is collected by venipuncture, Venipuncture blood collection materials and precision pipette, plus tips, and;
- Sterile gauze or cotton.

Storage

The test kit should be stored at 2 - 40 °C.

Shelf-life upon manufacture

24 months.

Warnings/limitations

Refer to current version of manufacturer’s instructions for use.

Prioritization for prequalification

Based on the results of the WHO product testing of malaria RDTs for Round 7 and eligibility criteria, **AdvDx Malaria Pf Rapid Malaria Ag Detection Test** was given priority for WHO prequalification assessment.

Dossier assessment

Advy Chemical Pvt Ltd. submitted a product dossier for **AdvDx Malaria Pf Rapid Malaria Ag Detection Test** as per the “*Instructions for compilation of a product dossier*” (PQDx_018 v1). The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

The manufacturer's responses to the nonconformities found during dossier screening and assessment findings were accepted on **05 December 2018**.

Commitments for prequalification

1. The manufacturer will provide a timeline by **8 June 2019** for the completion of the remaining commitments to Prequalification regarding the issues stated in WHO review of commitments letter dated 8 May 2019.
2. The manufacturer will provide the results of a study regarding the validation of 24 months of in-use stability for the assay buffer by **31 July 2020**.
3. The manufacturer will provide the results of the investigation of the real time stability after shipping by **30 November 2020**.

Based on the product dossier screening and assessment findings, the product dossier for **AdvDx Malaria Pf Rapid Malaria Ag Detection Test** meets WHO prequalification requirements.

Manufacturing site inspection

A comprehensive inspection was performed at the site(s) of manufacture (Plot No.A-334,336,338 & A-337 & 339 Road No. 25 & 26, Wagle Industrial Estate, Thane, India 400604) of **AdvDx Malaria Pf Rapid Malaria Ag Detection Test** on 2-4 October 2017 as per the “*Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics*” (PQDx_014). The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality.

The manufacturer's responses to the nonconformities found at the time of the inspection were accepted on 22 August 2018.

Based on the site inspection and corrective action plan review, the quality management system for **AdvDx Malaria Pf Rapid Malaria Ag Detection Test** meets WHO prequalification requirements.

Product performance evaluation

The seventh round of WHO product testing of RDTs for malaria antigen detection was completed in 2016. The product was evaluated against a Plasmodium falciparum cultured line panel, P. falciparum wild type parasite panel, P. vivax wild type parasite panel and a Plasmodium spp. negative panel. Thermal stability was assessed after 2 months of storage at elevated temperature and humidity, and a descriptive ease of use assessment was recorded.

Based on the demonstrated P. falciparum panel detection score (80.0% at 200 parasites/ μ l), false-positive rates (0.0% for clean negatives, 0.0% for P. vivax at 200 parasites/ μ l, 0.0% for P. vivax at 2000 parasites/ μ l) and invalid rate (0.0%), AdvDx Malaria Pf Rapid Malaria Ag Detection Test meets the current laboratory evaluation requirements for prequalification.

Summary performance characteristics	Panel detection score (%)	False positive rate (%)		Invalid rate (%)
	200 parasites/μl	200 parasites/μl	Clean negatives	
	Pf	Pv		
AdvDx Malaria Pf Rapid Malaria Ag Detection Test	80.0	0.0	0.0	0.0

Labelling

- 1. Labels**
- 2. Instructions for use**

1. Labels

Advy Chemical Pvt. Ltd
Advdx Malaria Pf_Carton_25Test

L 160 x W 117 X H 70mm

Pantone : - 7435 C
 Pantone : - 3025 C

25 Tests

ADV CHEMICAL
 ISO 9001 and
 EN ISO 13485 Certified

D

Store at 2 - 40°C
 For *In Vitro* Diagnostic Use Only

MALARIA Pf
 Rapid Malaria Ag Detection Test
 Detection of Pf(HRP-II) Antigen

**Adv
 Dx**

**Adv
 Dx**

MALARIA Pf
 Rapid Malaria Ag Detection Test
 Detection of Pf(HRP-II) Antigen

Store at 2 - 40°C
 For *In Vitro* Diagnostic Use Only

D

ADV CHEMICAL
 ISO 9001 and
 EN ISO 13485 Certified

25 Tests

Product Code: 00-DKM-RK-MAL-ADX-004-025

CONTENTS :
 Product Insert : 1 No.
 Test Device : 25 Nos.
 Sample Applicator : 25 Nos.
 Alcohol Swab : 25 Nos.
 Lancet : 25 Nos.
 Buffer Solution : 1 No.

Store at 2 - 40°C
 For *In Vitro* Diagnostic Use Only
 Disposal: Dispose all the samples and kit properly as per the instructions after test in accordance with GLP.



EC REP ALFABIO s.r.l. Lippensdri 21, B-6220 Hamme, Belgium



Materials required but not Provided :

- Timer
- Suitable eye protection
- New pair of disposable gloves
- Pen/pencil
- BioSafety sharps container
- Biohazard waste container (for potentially infectious waste)
- If whole blood is collected by venipuncture, Venipuncture blood collection materials and precision pipette, plus tips.
- Sterile gauze or cotton.

MALARIA Pf
 Rapid Malaria Ag Detection Test

0177RMA025IC-5

**Adv
 Dx**

MALARIA Pf
 Rapid Malaria Ag Detection Test
 Detection of Pf(HRP-II) Antigen

Store at 2 - 40°C
 For *In Vitro* Diagnostic Use Only

D

ADV CHEMICAL
 ISO 9001 and
 EN ISO 13485 Certified

25 Tests

Mfg. Lic. No. : KD 359

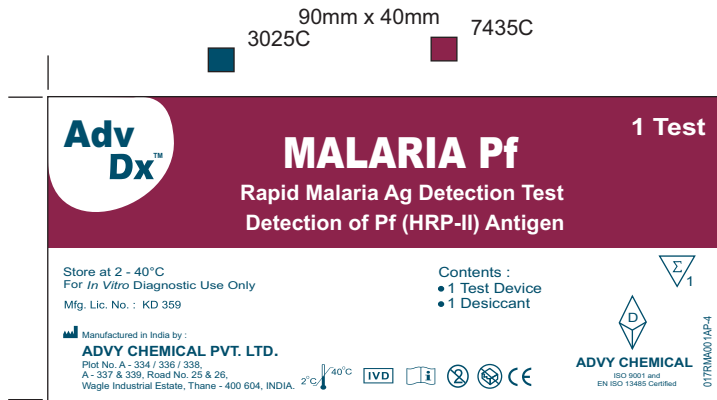
LOT
 MFG
 EXP
 M.R.P. ₹.
 (Incl. of all Taxes)

Manufactured in India by :
ADV CHEMICAL PVT. LTD.
 Plot No. A-334 / 336 / 338, Road No. 26,
 Wagle Industrial Estate, Thane - 400 604, INDIA.
 info@advychemical.com • www.advychemical.com
 ("Group of M/s. Bharat Serums and Vaccines Ltd.")
 Customer Care :
 Telephone No. +91 22 25830326
 E-mail : customerfeedback@advychemical.com

MALARIA Pf
 Rapid Malaria Ag Detection Test

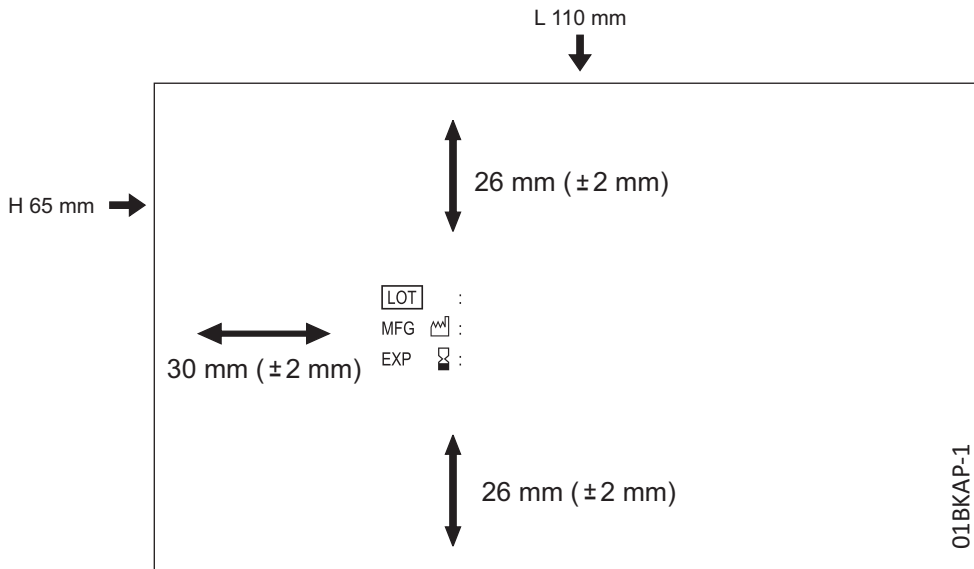
Advy Chemical Pvt. Ltd

Advdx Malaria Pf Pouch Label Front side



Advdx Malaria Pf Pouch Label Back side

Pouch Label Size: L 110 mm x H 65 mm

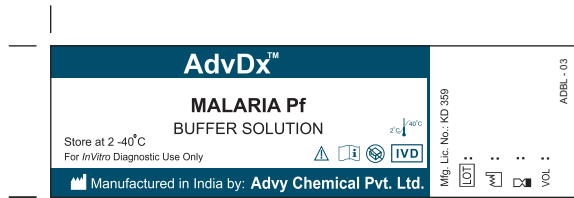


Advy Chemical Pvt. Ltd

Advdx Malaria Pf Pouch Label Front side

70mm x 20mm

■ 3025C ■ Black

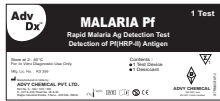


2. Instructions for use

AdvDx™ Malaria Pf Test Procedure

- FIRST, read carefully the Product Insert on how to use the AdvDx™ Malaria Pf kit.
- Now open the kit and look for the following.

1) Test device individually foil pouched with a desiccant.



2) Buffer Solution



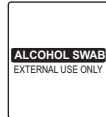
3) Product Insert

4) Sample dropper

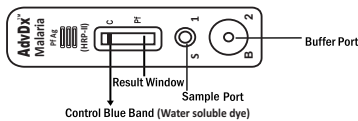
5) Lancet



6) Alcohol Swab



- Next, look at the expiry date at the back of the pouch. Use another kit, if expiry date has passed. Open the pouch and look for the following.



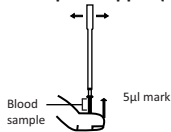
Note: Control blue band (water soluble dye) disappears as test runs and does not affect the test performance. Control blue band is visible in result window only before carrying out the test.

- Clean the patient's finger. The alcohol MUST be dried before pricking, or test may not work.
- Prick the patient's finger with the lancet to get blood (Wipe away the first drop of blood with sterile gauze or cotton)



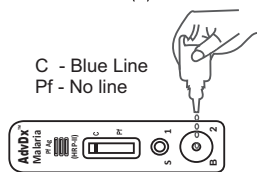
- Take 5µl blood using the Sample dropper Provided.

Sample Dropper (5µl)



Gently squeeze the disposable sample Dropper (5µl), immerse the open end into the blood sample and gently release to draw 5µl of blood up to the indicated mark with even pressure.

- Add 5µl blood into sample Port 1(S).
- Add Buffer Solution into the Buffer Part 2(B) of the test device



Add 4 drops (110µl ± 5µl) of buffer solution with even pressure

- INTERPRET TEST RESULTS AT THE END OF 20 MINUTES.

CAUTION : Do not read test after 30 minutes, since it may give incorrect results.

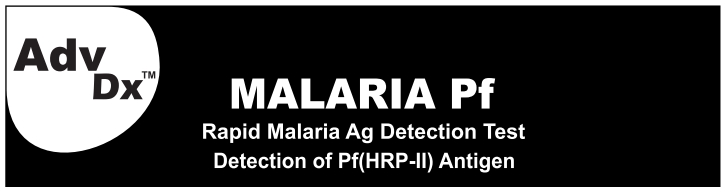


- Interpretation of Test Results.

Negative For Malaria	Positive For Malaria PF Positive
<p>Only one purple coloured Control band appears in the result window</p>	<p>In addition to purple coloured Control band 'Pf' band appears in the result window</p>

Invalid

<p>If the colour of the "C" control band is blue, then result considered Invalid</p>	<p>If the colour of the control band is Blue, then result considered Invalid</p>	<p>If no purple color Control band appears in the result window in spite of appearance of Purple band at Test line Pf the test is considered Invalid</p>	<p>If no purple Control band appears in the result window the test is considered Invalid</p>
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Ref. No.: 017RMA050KI-9

INTENDED USE

AdvDx™ Malaria Pf test kit is an in-vitro diagnostic Immunochromatographic assay for the qualitative detection of malaria infection caused by *Plasmodium falciparum* parasites in human. It detects HRP-II (Histidine Rich Protein-II) antigen of *Plasmodium falciparum* in whole blood specimens. It does not assess parasite densities. The test must be performed by trained professional user and not by lay users.

CLINICAL SIGNIFICANCE

Malaria is a serious, sometimes fatal, parasitic disease. It is characterized by fever with chills, anemia and is caused by Plasmodium parasite that is transmitted from one human being to another by the bite of infected Anopheles mosquitoes. Four species of the *Plasmodium parasite* are responsible for malaria infections in humans viz. *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Of these, *P. falciparum* and *P. vivax* are the most prevalent. Early detection and differentiation of malaria is of paramount importance due to incidence of cerebral malaria and drug resistance associated with falciparum malaria causing most of the morbidity and mortality worldwide.

PRINCIPLE

AdvDx™ Malaria Pf test utilizes the principle of Immuno-chromatography. It has the test strip coated with Monoclonal Anti-HRP-II (Test line Pf) which is specific to the histidine rich protein-II of *P. falciparum*. As the test sample flows through the membrane assembly of the device after addition of the buffer solution, the colored colloidal gold, and the anti- HRP-II antibody conjugate complexes with the lysed blood sample. The malarial antigens get immobilized on the respective test lines on the nitrocellulose membrane which leads to the formation of red/purple colored band/s. The unreacted conjugate continues to migrate and is subsequently immobilized at the control "C" region forming a red/purple band. The control band demonstrates that liquid has migrated, but does not demonstrate that the assay procedure has been followed correctly. The control band must appear to prove that sample or buffer have migrated though it does not indicate that the specimen is correct and the assay procedure is followed correctly.

MATERIALS PROVIDED

A AdvDx™ Malaria Pf kit contains the following items to perform the assay:

- AdvDx™ Malaria Pf Test Device individually foil pouched with a Desiccant.
- Sample dropper.
- Alcohol Swab.
- Lancet.
- Buffer Solution.
- Product Insert.

B Active ingredients of main components are:

- 1 test strip includes : Gold conjugate: Mouse monoclonal antibodies specific to Pf - HRP-II conjugated to colloidal gold, Control line : Goat anti-mouse IgG.
- Buffer Solution: Casein, Triton X-100 and Sodium azide as preservative.

Materials required but not provided

- Timer
- Suitable eye protection
- New pair of disposable gloves
- Pen/pencil
- BioSafety sharps container
- Biohazard waste container (for potentially infectious waste)
- If whole blood is collected by venipuncture, Venipuncture blood collection materials and precision pipette, plus tips.
- Sterile gauze or cotton.

PRECAUTIONS

- Read this Insert carefully before carrying out the test and instruction must be followed exactly to get accurate results.
- The Device is sensitive to humidity as well as to heat. Therefore take out the Device from sealed pouch just before carrying out the test.
- Store the AdvDx™ Malaria Pf kit at the temperature mentioned on the kit in a dry atmosphere.
- Do not use the kit after the expiration date.
- Do not mix reagents from different lots.
- For in-vitro diagnostic use only.
- Wear protective gloves while handling samples and wash hands thoroughly after performing the test.
- Dispose all the samples and kits properly as per the instructions after test in accordance with GLP.
- Do not pipette reagents or blood samples by mouth.
- Do not re-use the test.
- Buffer Solution: It contains Sodium azide as a preservative, in case of contact with skin, wash immediately. Wear gloves and eye protective.
- Do not use any other buffer than the buffer supplied within this kit.

13. Dispose any left-over specimen, tested device, lancet, sample dropper, empty buffer vial, used alcohol swab and used hand gloves as biohazard waste container in accordance with local regulation at the point of use. Silica pouch shall be opened and discarded in the local waste bin.

SPECIMEN COLLECTION, STORAGE & PRECAUTION

Specimen Required:

Capillary whole blood or whole blood with the following anticoagulants: EDTA, Citrate or Heparin

Collection by venipuncture

1. Collect the whole blood into the collection tube containing anticoagulant (EDTA, Citrate or Heparin) by venipuncture. Anticoagulant such as Heparin, EDTA and Citrate do not affect the test results.
2. If immediate testing is not possible then the samples may be stored at 2-8°C for upto 3 days. The samples should be brought to room temperature prior to use. Using samples kept for more than 3 days can cause non-specific reactions.

Collection with lancet:

1. Wear gloves.
2. Choose a finger for the finger prick:
 - Do not choose a finger that is swollen, bruised or scarred.
 - Preferably choose the 3rd or 4th finger of the hand the patient does not use to write.
3. Open the packaging of the alcohol swab. Take out the alcohol swab. Do not throw away the empty packaging (wrapper) but keep it aside.
4. Wipe the complete fingertip with the alcohol swab. Wait until the finger has completely dried (minimum 30 seconds).
5. Place the alcohol swab in the wrapper and set it aside (you will use it again to stop the bleeding after you collected the patient's blood).
6. While gently squeezing the disposable sample dropper (5µL) provided, immerse the open end in the blood drop and gently release draw 5µL of blood up to the indicated mark with even pressure.

Precaution:

1. Use separate sample dropper (5µL) fresh pipette tip for each sample to avoid cross-contamination and erroneous results.
2. Do not use any other specimen than whole blood.

TEST PROCEDURE:

1. Allow AdvDx™ Malaria Pf kit components and specimens to attain room temperature.
2. Open the pouch and take out the device from it.

Note: The device contains a blue control line made up of water soluble dye that disappears as the test runs. (Refer Point 3 of Text Procedure, Page No. 4)
3. Tighten the bottle cap of the buffer solution provided with the kit in the clockwise direction to pierce the dropper bottle nozzle. Hold the buffer bottle vertically this ensures that drops contain the correct volume of the buffer.
4. Evenly mix the anticoagulated blood sample by gently swirling and then dip the sample dropper (5µL) into the blood sample to draw 5µL of blood as shown in the figure.

OR

Incase of finger prick, clean the fingertip with an alcohol swab dry completely; prick the fingertip with a "Single Use Lancet". Wipe away the first drop of blood with sterile gauze cotton; with the help of the sample dropper (5µL), provided, draw 5µL of blood as shown in the figure.

5. Load 5µL of blood into the "Sample Port 1 (S)"
6. Add 4 drops (110 µL ± 5µL) of buffer solution into the "Buffer Port 2 (B)" on the test device.
7. Read the result at the end of 20 minutes.
8. Interpret the result. Refer to figure for interpretation of the result.

CAUTION: Do not read test after 30 minutes, since it may give incorrect results.

INTERPRETATION OF THE RESULTS:

Whole blood samples may cause red background to appear in the result window.

NEGATIVE:

Only the Purple-coloured control band appears. Negative result indicates no malaria antigens present in the blood sample, indicating no malaria infection or the number of malaria antigens the blood sample is below the detectable range.

POSITIVE:

1) Pf Positive: Two bands ("Pf" Test line and Purple-coloured "C" Control line) appears within the result window indicates the infection of *P. falciparum*.

The shade of colour / intensity of band may vary, but it should be considered positive whenever there is a faint line.

INVALID:

1) Absence of color band or blue color band at Control line (C) . with or without color band at the test line 'Pf' indicates the test is invalid. In this case, please repeat the test using a fresh device and follow the test procedure exactly.

STORAGE AND EXPIRATION:

1. AdvDx™ Malaria Pf Kit should be stored between 2°C to 40°C (36°F to 104°F). **Do Not freeze.**
2. The kit has a shelf-life of 24 months from the date of manufacture. The kit is stable until the expiration date marked on the product when stored as specified. The buffer is stable for 6 months after opening.

LIMITATIONS OF THE TEST:

1. AdvDx™ Malaria Pf kit is designed for primary screening of malaria infection by *P. falciparum*. Although the test is accurate in detecting HRP-II specific to *P. falciparum* in blood samples, a low incidence of false results can occur. Other clinically available tests are required if questionable results are obtained as with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. Malaria RDTs can give positive results after successful anti-malarial treatment. Therefore, the AdvDx™ Malaria Pf test kit is not recommended for monitoring response to anti-malarial treatment.

PERFORMANCE CHARACTERISTICS:

The AdvDx™ Malaria Pf Rapid test has been tested with Positive and negative clinical samples tested by microscopic examination of whole blood.

A. Sensitivity and Specificity

The sensitivity and Specificity for AdvDx™ Malaria Pf kit for *P. falciparum* malaria is 96.66% and 98.42% respectively. The performance of test was established by comparison with the results of microscopic examination of thick and thin films.

Result of AdvDx™ Malaria Pf	Reference Method (Microscopic examination)		Total
	Pf Positive	Pf Negative	
Positive	87	03	90
Negative	03	187	190
Total	90	190	280
% Sensitivity	% CI	% Specificity	% CI
96.66%	96.3 - 96.9	98.42%	98.3 - 98.5

A. Analytical Sensitivity (Limit of detection)

Analytical Sensitivity (LoD) of AdvDx™ Malaria Pf Rapid Malaria Ag Detection Test is 200 P/µl of *P. falciparum* samples and comparable to microscopic observations.

B. Analytical Specificity (Cross reactivity)

Analytical Specificity of AdvDx™ Malaria Pf Rapid Malaria Ag Detection Test studied with Infectious samples and different anticoagulants is 100%.

C. Precision:

Repeatability and Reproducibility of AdvDx™ Malaria Pf Rapid Malaria Ag Detection Test is 100%.

D. Residual Risk: None

REFERENCES:

1. World Health Organization – Geneva (2000). New perspectives Malaria diagnosis.
2. Perlmann, P and Troye-Blomberg, M. 2002. Malaria Parasites and disease. Malaria Immunology.
3. Malcolm, J.G., et al, 2002. Genome sequence of the human malaria parasite Plasmodium falciparum. Nature 419:498-511
4. Histidine Rich protein II: a novel approach to malaria drug sensitivity testing, Antimicrobial agents and Chemotherapy, June 2002, P. 1658–1664 Vol. 46, No.6.

DISCLAIMER:

Every precaution has been taken to ensure the diagnostic ability and accuracy of this product. This product is used outside of the control of the manufacturer and the distributor and the result may accordingly be affected by environmental factors and / or user error. A person who is the subject of the diagnosis should consult a doctor for further confirmation of the result.

WARNING:

The Manufacturers and Distributors of this product shall not be liable for any losses, liability, claims, costs, or damages whether direct or indirect or consequential arising out of or related to an incorrect diagnosis, whether positive or negative, in the use of this product.

For any complaint / query and suggestions: **Customer Care No. : +91 22 25830326**

ORDERING INFORMATION

PACK SIZE	PRODUCT CODE
25 Tests / Kit	00-DKM-RK-MAL-ADX-004-025
50 Tests / Kit	00-DKM-RK-MAL-ADX-004-050



Manufactured in India By:

ADY CHEMICAL PVT. LTD.

Plot No. A - 334 / 336 / 338,
A - 337 & 339, Road No. 25 & 26,
Wagle Industrial Estate, Thane - 400 604.
Email : info@advychemical.com
Website : www.advychemical.com

SYMBOL LEGENDS

Symbol	Explanation of symbol	Symbol	Explanation of symbol
	Consult instruction for use		Keep Dry
	Do not use if package is damaged		Batch code No.
	In vitro diagnostic device		Manufacturer
	Store at 2°C - 40°C		Date of Manufacture
	Keep away from sunlight		Use by (date or month of expiry)
	Do not re-use		Manufactured representative in the European community

Date Issued: 2019-04

