WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: One Step test for Malaria Pf HRP-II Ag
MERISCREEN Malaria Pf HRP-II Ag
WHO reference number: PQDx 0470-074-00

One Step test for Malaria Pf HRP-II MERISCREEN Malaria Pf HRP-II Ag with product codes RPWMPH-02, RPWMPH-03 and RPWMPH-04, manufactured by Meril Diagnostics Pvt. Ltd., RoW, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 15 November 2022.

Summary of WHO prequalification assessment for One Step test for Malaria Pf HRP-II Ag MERISCREEN Malaria Pf HRP-II Ag

	Date	Outcome
Prequalification listing	15 November 2022	listed
Dossier assessment	20 September 2022	MR
Desk assessment of quality management system	15-17 June 2020	MR
Product performance evaluation	1st quarter of 2020	MR

MR: Meets Requirements

Intended use:

According to the claim of intended use from Meril Diagnostics Pvt. Ltd., *"MERISCREEN Malaria Pf HRP-II Ag test kit is an in-vitro diagnostic immunochromatographic assay which provides a simple, rapid and in vitro qualitative detection of Plasmodium falciparum specific HRP II (Histidine Rich Protein II) in human capillary and/or venous whole blood. It does not require additional instrument.*
MERISCREEN Malaria Pf HRP-II Ag test is intended as an aid to the diagnosis of Plasmodium falciparum malarial infection.
INTENDED USER:
The test must be performed by a trained user (in either laboratory or point-of-care settings)."

Assay description:

According to the claim of assay description from Meril Diagnostics Pvt. Ltd., " *MERISCREEN Malaria Pf HRP-II Ag test utilizes the principle oflmmuno- chromatography. It has the test strip coated with Monoclonal Anti-HRP-II, which is specific to the histidine-rich protein-II of*

P. falciparum on the test line and GoatAnti Chicken lgY on the control line. As the test sample flows through the membrane of the device after the 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 a reddish-purple colored band. The unreacted conjugate continues to migrate and is subsequently immobilized at the control "C"·region forming a reddish-purple band. The control band must appear to demonstrate that liquid has migrated, but does not demonstrate that the assay procedure has been followed correctly."

Test kit contents:

Kit Contents	RPWMPH-02	RPWMPH-03	RPWMPH-04
Each pouch contains 1 test device and 1desiccant	10	25	50
Assay buffer bottle	1x3.0 ml	2x3.0 ml	4x3.0 ml
Specimen transfer devices-5 µL	10	25	50
Lancets	10	25	50
Alcohol swabs	10	25	50
Pack Insert	1	1	1

Items required but not provided:

- Disposable gloves
- Pen
- Timer
- Sharp box
- Non-sharps disposal container
- Venipuncture blood collection materials, precision pipette and pipette tips (if whole blood is collected by venipuncture)

Storage:

The test kit should be stored at 1-40 °C.

Shelf-life upon manufacture:

24 months.

Warnings/limitations:

Refer to the current version of the manufacturer's instructions for use attached to this public report.

Prioritization for prequalification:

Based on the established eligibility criteria, the One Step test for Malaria Pf HRP-II Ag MERISCREEN Malaria Pf HRP-II Ag was given priority for the WHO prequalification assessment.

Dossier assessment

Meril Diagnostics Pvt. Ltd. submitted a product dossier for One Step test for Malaria Pf HRP-II Ag MERISCREEN Malaria Pf HRP-II Ag as per the "*Instructions for compilation of a product dossier*" (PQDx_018 version 3). 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 20 September 2022.

Commitments for prequalification

1. The manufacturer will provide evidence regarding how it has been demonstrated that polyclonal antibodies used in the product design are free from encephalitis viruses. The manufacturer has provided the documentation. The documentation was under review when this Public report was published.
2. The manufacturer will provide evidence regarding a new LoD study report. The manufacturer has provided the documentation. The documentation was under review when this Public report was published.
3. The manufacturer will provide evidence regarding the analytical specificity study report. The manufacturer has provided the documentation. The documentation was under review when this Public report was published.

Based on the product dossier screening and assessment findings, the product dossier for the One Step test for Malaria Pf HRP-II Ag MERISCREEN Malaria Pf HRP-II Ag meets WHO prequalification requirements.

Manufacturing site inspection

A desk assessment of Meril Diagnostics Pvt. Ltd., located at D1-D3, Meril Park, Survey No 135/2/B & 174/2, Muktanand Marg, Chala, Vapi, 396191, India, was conducted from 15-17 June 2020. At the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of a product of consistent quality. Routine inspections of the Manufacturing site will be conducted with copies of the WHO Public Inspection Report (WHOPIR) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. Note that a WHOPIR reflects the information on the most current desk assessment performed at a manufacturing site for *in vitro* diagnostic products and gives a summary of the desk assessment findings.

Information on the most current desk assessment can be found at:
https://extranet.who.int/pqweb/inspection-services/prequalification-reports/whopirs-vitro-diagnostics

All published WHOPIRs are with the agreement of the manufacturer.

The manufacturer's responses to the nonconformities found at the time of the desk assessment were accepted on 13 July 2020.

Product performance evaluation

One Step test for Malaria Pf HRP-II Ag MERISCREEN Malaria Pf HRP-II Ag was evaluated in the 1st quarter of 2020 at the Centers for Disease Control and Prevention on behalf of WHO according to protocol PQDx_317, version 2.

One Step test for Malaria Pf HRP-II Ag MERISCREEN Malaria Pf HRP-II Ag was evaluated against a *Plasmodium falciparum* cultured line panel, *P. falciparum* wild type parasite panel, *P. vivax* wild-type parasite panel and a *P. falciparum* and *P.vivax* negative panel.

Performance characteristics	
	P. falciparum
Panel detection score at 200 parasites/µL (N=100)	84/100, 84.0%
False positive results	Negative specimens: 0/200, 0% Of which, clean negative specimens: 0/104, 0% *P. vivax* specimens at 200 and 2000 parasites/µL: 0/210, 0%
Invalid rate % (N= 1090)	0/1090, 0%
Inter-reader variability % (N=1010)	33/1010, 3.3%
The lowest concentration of HRP2 detected using the 1st WHO International standard for Pf antigens (NIBSC code: 16/376)*	31.3 IU/mL on both lots

** Not applicable for assays evaluated in WHO product testing of RDTs for malaria antigen detection*

Operational characteristics and ease of use

This assay does not require laboratory equipment and can be performed in laboratories with limited facilities or non-laboratory settings.

The assay was found easy to use by the operators performing the evaluation.
However, the operators reported that the buffer container top/cap must be carefully twisted to puncture the buffer container tip. If the top/cap is not completely flush when twisting, then the puncture device within the top/cap will break and not puncture the buffer container tip (another top/cap from another bottle from the test kit can be used to repeat). The operators also noted that 96/1090 (8.8%) tests showed anomalies, including incomplete clearing (n=94) and red background (n=2).

Key operational characteristics	
Specimen type and volume	5 µL of capillary whole blood or venous whole blood collected in a tube containing anticoagulants, including heparin, EDTA or Sodium Citrate
Number of steps*	2 steps in total 1 steps with precision pipetting *(only for venous whole blood)*
Time to result	20 minutes
Endpoint stability (interval)	10 minutes (the test can be read between 20 and 30 minutes after the addition of the buffer)
Internal QC	Yes, reagent addition control

** Definition: each action required to obtain a result (excluding specimen collection, device preparation – opening the pouch), e.g. for RDTs: add specimen, add buffer (2 steps).*

Based on these results, the performance evaluation for the One Step test for Malaria Pf HRP-II Ag MERISCREEN Malaria Pf HRP-II Ag meets the WHO prequalification requirements.

Labelling

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

1. Labels

1.1 Labels for product code RPWMPH-02

Product Box Label

REF **RPWMPH-02**

IVD One Step Test for Malaria Pf HRP-II Ag

MERISCREEN Malaria Pf HRP-II Ag

Σ 10 For *in-Vitro* diagnostics use only

KIT CONTAINS

1. 10 Individually Packed Test Devices
2. 10 Specimen Transfer Devices-5µl
3. 1 Assay Buffer (3.0ml)
4. 1 Product Insert
5. 10 Alcohol swabs
6. 10 Lancets

One Step, Rapid test for the detection of *Plasmodium falciparum* specific HRP-II (Histidine Rich Protein-II) in human capillary and/or venous whole blood.

Meril Diagnostics

Mfg.Lic.No.: MFG/MD/2018/000029

LOT XXXXXX

YYYY.MM.DD

YYYY.MM.DD

1°C 40°C

Meril Diagnostics Pvt. Ltd.
Second Floor, D1-D3, Meril Park, Survey No.135/2/B &174/2, Muktanand Marg, Chala, Vapi-396191, Gujarat, India. Tel:+91-260-2408000, Fax:+91-260-2408025, E-mail:diagnostics@merillife.com, www.merillife.com

Aluminum Pouch Label

REF **RPWMPH-02**

IVD One Step Test for Malaria Pf HRP-II Ag

Σ 1 **MERISCREEN Malaria Pf HRP-II Ag**

For *in-Vitro* diagnostics use only

Meril Diagnostics

Store at 1ºC - 40ºC

Do not open the foil pouch until it attains room temperature

1°C 40°C

Mfg.Lic.No.: MFG/MD/2018/000029

LOT XXXXXX

YYYY.MM.DD

YYYY.MM.DD

Meril Diagnostics Pvt. Ltd.
Second Floor, D1-D3, Meril Park, Survey No.135/2/B &174/2, Muktanand Marg, Chala, Vapi-396191, Gujarat, India. Tel:+91-260-2408000, Fax:+91-260-2408025, E-mail:diagnostics@merillife.com, www.merillife.com

Assay Buffer Bottle Label

REF **RPWMPH-02**

IVD One Step Test for Malaria Pf HRP-II Ag

MERISCREEN Malaria Pf HRP-II Ag

Meril Diagnostics

1°C 40°C

Assay Buffer (Vol. 3.0ml)

Mfg.Lic.No.: MFG/MD/2018/000029

LOT XXXXXX

YYYY.MM.DD

YYYY.MM.DD

Meril Diagnostics Pvt. Ltd.
Second Floor, D1-D3, Meril Park, Survey No.135/2/B &174/2, Muktanand Marg, Chala, Vapi-396191, Gujarat, India. Tel:+91-260-2408000, Fax:+91-260-2408025, E-mail:diagnostics@merillife.com, www.merillife.com

1.2 Labels for product code RPWMPH-03

Product Box Label

REF **RPWMPH-03**

IVD One Step Test for Malaria Pf HRP-II Ag

MERISCREEN Malaria Pf HRP-II Ag

For *in-Vitro* diagnostics use only

Σ 25

KIT CONTAINS

1. 25 Individually Packed Test Devices
2. 25 Specimen Transfer Devices-5µl
3. 2 Assay Buffer (3.0ml)
4. 1 Product Insert
5. 25 Alcohol swabs
6. 25 Lancets

One Step, Rapid test for the detection of *Plasmodium falciparum* specific HRP-II (Histidine Rich Protein-II) in human capillary and/or venous whole blood.

Meril Diagnostics

Mfg.Lic.No.: MFG/MD/2018/000029

LOT XXXXXX

YYYY.MM.DD

YYYY.MM.DD

1°C – 40°C

Meril Diagnostics Pvt. Ltd.
Second Floor, D1-D3, Meril Park, Survey No.135/2/B &174/2, Muktanand Marg, Chala, Vapi-396191, Gujarat, India. Tel:+91-260-2408000, Fax:+91-260-2408025, E-mail:diagnostics@merillife.com, www.merillife.com

Aluminum Pouch Label

REF **RPWMPH-03**

IVD One Step Test for Malaria Pf HRP-II Ag

MERISCREEN Malaria Pf HRP-II Ag

Σ 1

For *in-Vitro* diagnostics use only

Meril Diagnostics

Store at 1ºC - 40ºC

Do not open the foil pouch until it attains room temperature

1°C – 40°C

Mfg.Lic.No.: MFG/MD/2018/000029

LOT XXXXXX

YYYY.MM.DD

YYYY.MM.DD

Meril Diagnostics Pvt. Ltd.
Second Floor, D1-D3, Meril Park, Survey No.135/2/B &174/2, Muktanand Marg, Chala, Vapi-396191, Gujarat, India. Tel:+91-260-2408000, Fax:+91-260-2408025, E-mail:diagnostics@merillife.com, www.merillife.com

Assay Buffer Bottle Label

REF **RPWMPH-03**

IVD One Step Test for Malaria Pf HRP-II Ag

MERISCREEN Malaria Pf HRP-II Ag

Meril Diagnostics

1°C – 40°C

Assay Buffer (Vol. 3.0ml)

Mfg.Lic.No.: MFG/MD/2018/000029

LOT XXXXXX

YYYY.MM.DD

YYYY.MM.DD

Meril Diagnostics Pvt. Ltd.
Second Floor, D1-D3, Meril Park, Survey No.135/2/B &174/2, Muktanand Marg, Chala, Vapi-396191, Gujarat, India. Tel:+91-260-2408000, Fax:+91-260-2408025, E-mail:diagnostics@merillife.com, www.merillife.com

1.3 Labels for product code RPWMPH-04

Product Box Label

REF **RPWMPH-04**

IVD

One Step Test for Malaria Pf HRP-II Ag

MERISCREEN Malaria Pf HRP-II Ag

Σ 50 For *in-Vitro* diagnostics use only

KIT CONTAINS

1. 50 Individually Packed Test Devices
2. 50 Specimen Transfer Devices-5µl
3. 4 Assay Buffer (3.0ml)
4. 1 Product Insert
5. 50 Alcohol swabs
6. 50 Lancets

One Step, Rapid test for the detection of *Plasmodium falciparum* specific HRP-II (Histidine Rich Protein-II) in human capillary and/or venous whole blood.

Meril Diagnostics

Mfg.Lic.No.: MFG/MD/2018/000029

LOT XXXXXX

YYYY.MM.DD

YYYY.MM.DD

1°C 40°C

Meril Diagnostics Pvt. Ltd.
Second Floor, D1-D3, Meril Park, Survey No.135/2/B &174/2, Muktanand Marg, Chala, Vapi-396191, Gujarat, India. Tel:+91-260-2408000, Fax:+91-260-2408025, E-mail:diagnostics@merillife.com, www.merillife.com

Aluminum Pouch Label

REF **RPWMPH-04**

IVD

Σ 1

One Step Test for Malaria Pf HRP-II Ag

MERISCREEN Malaria Pf HRP-II Ag

For *in-Vitro* diagnostics use only

Store at 1ºC - 40ºC

Do not open the foil pouch until it attains room temperature

1°C 40°C

Meril Diagnostics

Mfg.Lic.No.: MFG/MD/2018/000029

LOT XXXXXX

YYYY.MM.DD

YYYY.MM.DD

Meril Diagnostics Pvt. Ltd.
Second Floor, D1-D3, Meril Park, Survey No.135/2/B &174/2, Muktanand Marg, Chala, Vapi-396191, Gujarat, India. Tel:+91-260-2408000, Fax:+91-260-2408025, E-mail:diagnostics@merillife.com, www.merillife.com

Assay Buffer Bottle Label

REF **RPWMPH-04**

IVD

One Step Test for Malaria Pf HRP-II Ag

MERISCREEN Malaria Pf HRP-II Ag

Meril Diagnostics

1°C 40°C

Assay Buffer (Vol. 3.0ml)

Mfg.Lic.No.: MFG/MD/2018/000029

LOT XXXXXX

YYYY.MM.DD

YYYY.MM.DD

Meril Diagnostics Pvt. Ltd.
Second Floor, D1-D3, Meril Park, Survey No.135/2/B &174/2, Muktanand Marg, Chala, Vapi-396191, Gujarat, India. Tel:+91-260-2408000, Fax:+91-260-2408025, E-mail:diagnostics@merillife.com, www.merillife.com

1.4 Alcohol swab label

1.5 Sterile lancet label

1.6 Specimen transfer device

2. Instructions for use[1]

[1] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

PERFORMANCE SPECIFICATIONS:

A. Sensitivity and specificity:

In-house Testing:

228 P. falciparum positive specimens including positive samples were tested with MERISCREEN Malaria Pf HRP-II Ag to evaluate the diagnostic sensitivity of MERISCREEN Malaria Pf HRP-II Ag kit. 1000 *P. Falciparum* negative specimens were tested with MERISCREEN Malaria Pf HRP-II Ag to evaluate the Diagnostic Specificity of MERISCREEN Malaria Pf HRP-II Ag kit. The status of the sample was determined by microscopic examination:

Site	Specimen type	Diagnostic Sensitivity/ Diagnostic Specificity	% CI
Diagnostic Sensitivity	228 Pf positive venous whole blood specimens	98.25%	95.57% to 99.52%
Diagnostic Specificity	138 Pf negative capillary whole blood specimens	100.00%	97.36% to 100.00%
	70 Pf negative venous whole blood specimens	99.77%	99.16% to 99.97%

Testing at two different sites:

At Site 1, 330 P. falciparum positive specimens from population including pregnant women, children and 835 Plasmodium negative specimens were tested on MERISCREEN Malaria Pf HRP-II Ag kit to evaluate diagnostic sensitivity & specificity. 330 P. falciparum positive specimens include 260 specimens which were of capillary whole blood and 70 specimens were of venous whole blood. These specimens include 46 Pf positive specimens from children population and 33 Pf positive specimens from pregnant women. 835 Plasmodium negative specimens include 582 specimens which were collected as capillary whole blood and 253 specimens were collected as venous whole blood. These specimens include 60 negative specimens from pregnant women population and 102 negative specimens At Site 2, 92 P. falciparum positive specimens and 170 Plasmodium negative specimens were tested on MERISCREEN Malaria Pf HRP-II Ag kit to evaluate diagnostic sensitivity & specificity. 92 P. falciparum positive specimens include 52 specimens of capillary whole blood and 40 specimens of venous whole blood. These specimens include 15 Pf positive specimens from children population and 11 Pf positive specimens from pregnant women. 170 Plasmodium negative specimens include 150 specimens collected as capillary whole blood and 20 specimens collected as venous whole blood. These negative specimens include 40 specimens collected from pregnant women population and 34 specimens collected from children.

Site	Specimen type	Diagnostic Sensitivity	% CI
Site 1	260 Pf positive capillary whole blood specimens	100.00%	98.59% to 100.00%
	70 Pf positive venous whole blood specimens	100.00%	94.87% to 100.00%
Site 2	52 Pf positive capillary whole blood specimens	100.00%	93.15% to 100.00%
	40 Pf positive venous whole blood specimens	100.00%	91.19% to 100.00%

Site	Specimen type	Diagnostic Specificity	% CI
Site 1	582 Pf negative capillary whole blood specimens	100.00%	99.37% to 100.00%
	253 Pf negative venous whole blood specimens	100.00%	98.55% to 100.00%
Site 2	150 Pf negative capillary whole blood specimens	100.00%	97.57% to 100.00%
	20 Pf negative venous whole blood specimens	100.00%	83.16% to 100.00%

Symbols used on Meril Diagnostics labels:

REF Catalogue No. | LOT Batch No. | Consult instruction for use
Manufacturer | Expiry date | For single use only do not reuse
Manufacturing date | Keep dry | Keep away from direct sunlight
Storage temperature | Sufficient for | Do not use if box open or damaged
IVD In Vitro Diagnostics | Caution

B. Analytical sensitivity (Limit of detection):

The analytical sensitivity of One Step test for MERISCREEN Malaria Pf HRP-II Ag for *P. falciparum* is ≥50 parasites/µl.

C. Analytical specificity (Cross reactivity):

To evaluate the interference of One Step test for MERISCREEN Malaria Pf HRP-II Ag test kit with known relevant interfering specimens, the haemolytic specimens, rheumatoid factors-contained specimens and lipaemic, icteric specimens were investigated. In this study, the performance of the MERISCREEN Malaria Pf HRP-II Ag test kit was not affected by interfering substances such as bilirubin, triglyceride, acetaminophen, total protein, vitamin B12, sodium azide, thimerosol, alcohol/ethanol, hemoglobin, lipids, aspirin, caffeine, Ibuprofen, ABD PLUS Suspension 10 ml, EFAVIR 200 Efavirenz Capsules IP 200 mg, AkuriT-4 900 mg, and cross-reacting infections and antibodies such as Elevated IgG and IgM, recipients of multiple blood transfusion, pregnant women (including multiparus), Acute Hep A infection, Yellow Fever Virus, Post-immunization Measles, Influenza A, Influenza B, Rheumatoid Arthritis, Hepatitis B, Hepatitis C, Syphilis, HIV, Dengue, P. vivax, Salmonella typhi, Wuchereria bancrofti and Brugia malayi, Tick Borne Encephalitis, Human Anti-Mouse Antibody (HAMA), Systemic Lupus Erythematous (SLE), Anti-Nuclear Antibodies (ANA). The performance of the One Step test for MERISCREEN Malaria Pf HRP-II Ag test kit is not affected by interfering substances/cross reactive agents.

D. Precision:

The assay is 100% precise within and between runs when tested with same samples.

BIBLIOGRAPHY:

1. Carter, R. and Mendis, K.N. (2002). Evolutionary and Historical aspects of the burden of malaria. *Clin. Microbial.,* 15(4): 564-594.
2. Chandler, J. *et al.,* (2000). The place of gold in rapid tests. *IVD Technology*, 7(2): 37-49.
3. Moody, A. (2002). Rapid Diagnostics tests for malaria parasites. *Clin. Microbiol.*, 15(1): 66-78.
4. Murray, C. *et al.,* (2008). Update on Rapid Diagnostics Testing for malaria. *Clin. Microbiol.*, 21(1): 97-110.
5. Robinson, N. (2002). Immunogold conjugation for IVD applications. *IVD Technology*, 8(3): 33-36.
6. Weiss, A. (1999). Concurrent engineering for lateral flow diagnostics. *IVD Technology*, 5(7): 48-57.
7. Gamboa, D. *et al.,* (2010). A large proportion of P. falciparum isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. *PLoS One*, 5(1): e8091.
8. Gillet, P. *et al.,* (2011). Prozone in malaria rapid diagnostics tests: how many cases are missed? *Malar J.,* 10: 166.
9. Jacobs, J. (2014). Harmonization of malaria rapid diagnostic tests: best practices in labelling including instructions for use. *Malar J*, 13: 505.
10. WHO Guidelines for malaria – 18 Feb, 2022: World Health Organization (WHO).
11. Malaria Rapid Diagnostic Test Performance: Summary results of WHO product testing of malaria RDTs: round 1-8 (2008–2018).
12. WHO (World Health Organization) Guidelines on Drawing Blood: Best practices in phlebotomy.

Product disclaimer:

Every precaution has been taken to ensure the diagnostic ability and accuracy of this product. The product is used outside of the control of the Manufacturer and 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.

IFU/RPWMPH02/04
Date: 27/10/2022

One Step Test for Malaria Pf HRP-II Ag

MERISCREEN Malaria Pf HRP-II Ag

Meril Diagnostics

IVD For in vitro diagnostic use
Read this pack insert thoroughly before use

Product Code:	Pack Size:
RPWMPH - 02	10T
RPWMPH - 03	25T
RPWMPH - 04	50T

INTENDED USE:

MERISCREEN Malaria Pf HRP-II Ag test kit is an in-vitro diagnostic immunochromatographic assay which provides a simple, rapid and in vitro qualitative detection of Plasmodium falciparum specific HRP II (Histidine Rich Protein II) in human capillary and/or venous whole blood. It does not require additional instrument.

MERISCREEN Malaria Pf HRP-II Ag test is intended as an aid to the diagnosis of Plasmodium falciparum malarial infection.

INTENDED USER:

The test must be performed by a trained user (in either laboratory or point-of-care settings).

INTRODUCTION:

Malaria is a serious protozoan parasitic disease characterized by fever, chills and anemia[1,3,7,8]. It is caused by a bite of female Anopheles mosquito resulting in transmission of malarial parasites to human[1, 3, 7, 8]. There are four major types of human malaria: *P. falciparum, P.vivax, P.ovale* and *P.malariae*[1, 3, 7, 8, 10]. When infected, the parasites - sporozoites migrate to the liver where they mature and then are released to the blood stream of human infecting the red blood cells. Malaria infection occurs in more than 92 countries[8] worldwide but is mostly prevalent in sub-Saharan Africa and South East Asia [8]. It is estimated that there are over 219 million clinical cases and about 0.4 million malaria-caused deaths per year.

WHO recommends parasitological confirmation of malaria in all settings by quality-assured diagnosis before treatment is started. A diagnosis of malaria can be confirmed rapidly by good-quality microscopy or with a good-quality malaria antigen-detecting RDT [2, 3, 7, 8] for *Plasmodium falciparum* and non-*falciparum* infections.

PRINCIPLE:

MERISCREEN Malaria Pf HRP-II Ag test utilizes the principle of Immuno-chromatography [2, 3, 4,5]. It has the test strip coated with Monoclonal Anti-HRP-II which is specific to the histidine rich protein-II of *P. falciparum* on the test line and Goat Anti Chicken IgY on the control line. As the test sample flows through the membrane 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 reddish purple colored band. The un-reacted conjugate continues to migrate and is subsequently immobilized at the control “C” region forming a reddish purple band. The control band demonstrates that liquid has migrated but does not demonstrate that the correct specimen type or volume has been added[2,3,4, 6,11]

MATERIALS PROVIDED:

Kit Contents	RPWMPH-02	RPWMPH-03	RPWMPH-04
Each pouch containing 1 test device, 1 desiccant	10	25	50
Assay buffer bottle	1x 3.0ml	2 x 3.0ml	4 x 3.0ml
Specimen transfer devices-5 µL	10	25	50
Lancets	10	25	50
Alcohol swabs	10	25	50
Pack Insert	1	1	1

MATERIALS REQUIRED BUT NOT PROVIDED:

- Disposable gloves
- Pen
- Timer
- Sharp box
- Non-sharps disposal container
- Venipuncture blood collection materials, precision pipette and pipette tips (if whole blood is collected by venipuncture)

TEST KIT STORAGE AND STABILITY:

- Store the kit between 1-40 °C
- Do not store the kit in the freezer
- Protect the kit from heat and humidity
- The kit including assay buffer has a shelf life of 24 months from the date of manufacture. The test kit is stable until the expiration date marked on the kit box and/or the packaging of individual components when stored as specified.
- Once opened the Test device, it gives accurate results within 24 hours, but it should be used immediately.
- In case, the desiccant pouch has changed colour from blue to light pink or colourless, the device should not be used. The unopened test device pouch is stable up to the expiration date printed on the sealed pouch.

SPECIMEN REQUIRED:

- Capillary blood or venous blood with the following anticoagulants: EDTA, Heparin, Sodium Citrate[10,11,12].
- Time between collection and analysis:
  Capillary: immediately [12].
  Venous: immediately. If immediate testing is not possible, store the whole blood specimen at 2-8°C for maximum 72 hours (3 days).
- Venous whole blood specimens can be stored at -80 °C and that only one freeze-thaw cycle is allowed.

WARNING AND PRECAUTIONS:

1. For *in vitro* diagnostic use only.
2. Read the instructions carefully before performing the test. The instruction must be followed exactly to get accurate results.
3. Apply standard biosafety precautions for handling and disposal of potentially infective material/used kit/expired kit.
   - Handle all specimens as potentially infectious.
   - Wear gloves while handling specimens and performing the test.
   - Avoid splashing and aerosol formation.
   - Clean up spills thoroughly using an appropriate disinfectant.
4. The buffer contains sodium azide as a preservative which may be toxic if ingested. When disposed off through a sink, flush with large quantities of water.
5. Do not use any other buffer than the buffer supplied within this kit.
6. Do not use the RDT kit beyond the expiration date.
7. Do not use if the packaging is damaged.
8. Do not use any other specimen than whole blood.
9. Do not use if the product has been exposed to excessive heat or humidity.
10. Perform the test immediately after opening of the device packaging.
11. Do not re-use the test.
12. Do not touch the nitrocellulose part of the device. Finger print or scratch on nitrocellulose membrane may give erroneous results.
13. Do not use the lancet if the seal is broken.
14. Do not touch the tip of buffer bottle, it might contaminate buffer. Allow all reagents and specimen(s) to attain room temperature (15°C to 30°C) before use.
15. Do not smoke, eat or drink while handling specimens and performing a test.
16. Do not use haemolysed specimen for testing.
17. Contamination of specimen transfer devices and/or reagents can lead to inaccurate results.
18. Do not exchange or pool the components or reagents from different lots. This may lead to erroneous or invalid results.
19. Use of heparin, EDTA and sodium citrate anticoagulants do not affect the test results. Use of other anticoagulants have not been validated. Their use may affect the test result.
20. Use a new disposable specimen transfer device for each specimen in order to avoid cross contamination of specimens, which could produce erroneous results.
21. Discard the lancet or alcohol swab if package is pierced or damaged. The item may no longer be sterile; there is risk of infection if used.
22. Specimens frozen at -80 °C must not be used after one freeze-thaw

Meril Diagnostics Pvt. Ltd., Second Floor, D1-D3, Meril Park, Survey No. 135/2/B & 174/2, Muktanand Marg, Chala, Vapi-396191, Gujarat, India.
Tel.: +91-260-240 8000, Fax: +91-260-240 8025, E-mail: diagnostics@merillife.com, Web: www.merillife.com

cycle.

23. In-case No background clearing due to excessive specimen or buffer addition or no control line appearing, discard the test device and use the new test device.

SPECIMEN COLLECTION AND HANDLING:

Collection by venipuncture: [10,11,12]

1. Using venipuncture, draw whole blood into the collection tube (containing anticoagulants including heparin, EDTA or Sodium Citrate).
2. Whole blood specimens should be tested as soon as possible after collection. If whole blood specimens cannot be tested immediately, it must be refrigerated at 2-8°C and tested within 3 days of collection.
3. Do not use a blood specimen stored for more than 3 days; it can cause a nonspecific reaction.
4. Bring blood specimens to room temperature (15-30°C) prior to use.

Collection through finger prick: [10,11,12].

1. Clean the area to be lanced with an alcohol swab.
2. Squeeze the fingertip then prick the lateral side of the finger with the sterile lancet provided. Immediately, safely dispose of the lancet.
3. Wipe away the first drop of blood with sterile gauze or cotton.
4. Using a new disposable specimen transfer device provided, dip the circular end of the specimen transfer device into the blood specimen.
5. The specimen collected must be used immediately. The specimen collected cannot be stored.

TEST PROCEDURE:

1. Bring the test components, reagents and specimens to room temperature (15-30°C), if refrigerated.
2. When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.
3. Add capillary or venous whole blood sample into sample well (S) by using specimen transfer device or micropipette.
4. Dispose off used specimen transfer device as a bio-hazard waste.
5. Add four drops(100µl ± 10 µl) of the Assay Buffer to the buffer well (B).
6. Read the results after 20 minutes.

PICTORIAL REPRESENTATION OF PROCEDURE:

Preparation:

1. Opened Kit Contains:

I. Test device individually packed with a desiccant in aluminum pouch

II. Test device

III. Desiccant

IV. Assay buffer bottle

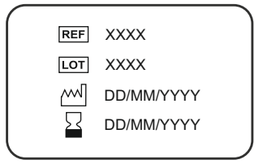
V. Specimen transfer device - 5 µL (Inverted Cup)

VI. Lancet

VII. Alchohol Swab

VIII. Pack Insert

2. Check the expiration date of the test. If expired, do not use it but take another test from an unexpired kit:



3. Open the test device packaging and check the desiccant. If Desiccant shows saturation (color changed from blue to pink/white), throw away the test device and take another test device packaging:

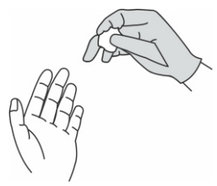
Blue: Use Device

White or Pink: Throw the dewice, Use new Device

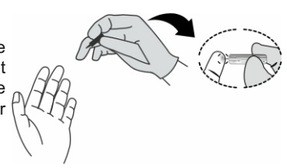
Test Procedure:

1. Capillary Whole Blood from finger prick:

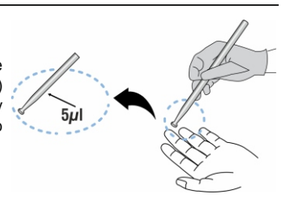
I. Wipe the complete fingertip to be lanced with the alcohol swab.



II. Prick the lateral side of the finger with the sterile lancet provided. Then, safely dispose off the lancet immediately after using.

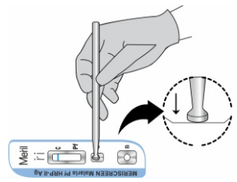


III. Using a new disposable specimen transfer device (5 µl) provided, collect 5 µl of blood by dipping the circular end of it into the whole blood drop.



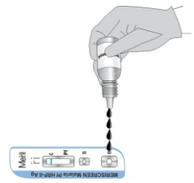
IV. Dispense 5 µl of drawn whole blood specimen into the specimen well (marked "S").

Use of specimen transfer device: Let the circular end of it should touch the pad, then press down gently.



V. Dispense 4 Drops(100µl ± 10 µl) of assay buffer in buffer well (marked "B").

Hold the buffer bottle vertically – this ensures that the drops contain the correct volume of buffer

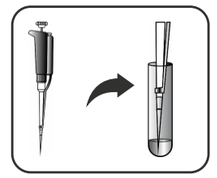


VI. Interpret the results after 20 minutes.

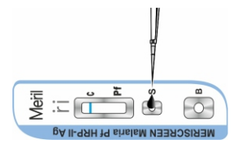
Do not read test results after 30 minutes.

2. Venous Whole blood from Venipuncture:

I. Draw 5 µl of specimen using micropipette

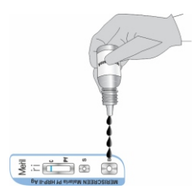


II. Dispense 5 µl of samples into the specimen well (marked"S").



III. Dispense 4 Drops (100µl ± 10 µl) of assay buffer in buffer well (marked "B").

Hold the buffer bottle vertically – this ensures that the drops contain the correct volume of buffer



IV. Interpret the results after 20 minutes.

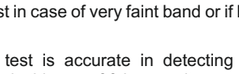
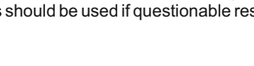
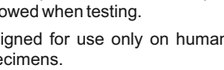
Do not read test results after 30 minutes.

INTERPRETATION OF THE TEST RESULT: [11]

1. After 20 but no later than 30 minutes: compare the test lines with the presentation in the table below.
2. Where possible, have the results confirmed by a second reader within this time frame.
3. Line intensities may vary from faint to strong intensity. Consider also a faint test line as a positive result.

   Note: Test line of any Intensity (light to dark) should be considered positive.
4. Record the test results as noted in the table below. Consult the national guidelines for malaria case management to complement the table below.

   Note: The faint blue line at "Control" position is always visible before testing. This faint blue line should not be interpreted as Control line during result interpretation.

Lines that you see	Picture / Drawing	Record the following result, take the following action
Faint Blue line at Control position even after addition of samples and assay buffer		Invalid. Take a new device and repeat the test.
NO Pink - Purple Line at 'C' (=control)		Invalid. Take a new device and repeat the test.
Pink - Purple Line at 'C' and **NO** other line		Negative
Pink - Purple Line at 'C' and **'Pf'**		Positive for *P. falciparum*

LIMITATIONS:

- The test procedure, precautions and interpretation of result for this test must be followed when testing.
- The test is designed for use only on human capillary and venous whole blood specimens.
- The following anticoagulants have been validated for use with this test: heparin, EDTA and citrate.
- The test results must not be the sole basis for clinical management. As with all diagnostics, the test results must be correlated with other clinical and epidemiological findings.
- This test may still produce a positive result after successful anti-malarial treatment. Therefore, its use is not recommended for monitoring a response to anti-malarial treatment.
- The test may produce a false positive result for a patient with acute schistosomiasis, a high level of rheumatoid factor (4000-5000 IU/mL) or presence of human anti-mouse IgG antibody (3000-3500 ng/mL) and use of wrong buffer.
- Hook effect due to very high parasite densities i.e., ≥24,583 parasites/µl for *P. falciparum.* Repeat the test by using different dilutions of same sample.
- This assay cannot be used for the diagnosis of infection by other type of malarial parasites *(P. vivax, P.malariae, P.ovale* or *P.knowlesi)* [10,11]
- A negative result at any time does not preclude the possibility of exposure or infection.
- Repeat the test in case of very faint band or if have any doubt for test band.
- Although the test is accurate in detecting HRP-II specific to P. falciparum low incidence of false results may occur. Other clinically available tests should be used if questionable results are obtained.