WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag
WHO reference number: PQDx 0294-074-00

One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag with product codes **MFLRPD-05, MFLRPD-02**, **MFLRPD-03 and MFLRPD-04** manufactured by **Meril Diagnostics Pvt. Ltd.**, **CE-mark regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 09 November 2018.

Summary of WHO prequalification assessment for MERISCREEN Malaria Pf/Pv Ag

	Date	Outcome
Prequalification listing	09-Nov-2018	listed
Dossier assessment	13-Sep-2018	MR
Site inspection(s) of quality management system	15-17 -Jun-2020	MR
Product performance evaluation	2016	MR

MR: Meets Requirements

Report amendments and/or product changes

This public report has since been amended. Amendments may have arisen because of changes to the prequalified product for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

Version	Summary of amendment	Date of report amendment
2.0	1. Fulfillment of prequalification commitments and addition of labels for sterile lancets and alcohol swabs. Addition of new pack sizes of 25 tests and 50 tests to the existing pack size of 30 tests. Product codes for these two new pack sizes are: MFLRPD-03 (25T) and MFLRPD-04 (50T). 2. Change in regulatory version i.e., RoW (rest-of world) version to CE mark version of product codes MFLRPD-02, MFLRPD-03, MFLRPD-04).	30 -Mar-2020

3.0	1. Addition of pack size (10T) with product code, MFLRPD-05 to serve the requirement of various tenders across the globe. 2. Change in Labels and Pack insert of MERISCREEN Malaria Pf/Pv Ag kit i.e., incorporation of information of CE mark of lancets and alcohol swabs is made due to request from European Representative of Meril Diagnostics i.e., Obelis S.A. Belgium.	24-May-2021
4.0	Changed the telephone and fax numbers of the Manufacturer in the labelling of MERISCREEN Malaria Pf/Pv Ag.	15-Nov-2021

Intended use:

According to the intended use claimed by the manufacturer "***One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag*** *test kit is an in vitro diagnostic immunochromatographic assay for the qualitative detection of infections with Plasmodium falciparum and P. vivax parasites causing malaria in human whole blood specimens. It does not assess parasite densities. It assists trained users (in either laboratory or point-of-care settings):*

- *in detecting Plasmodium falciparum and P. vivax infections*
- *to differentiate infection between Plasmodium falciparum histidine-rich protein II (Pf-HRP-II) and Plasmodium vivax Plasmodium lactate dehydrogenase (Pv-pLDH)*

The assay is intended for trained users and for an initial screening as well as an aid to the diagnosis of malaria infection.
Note: Malaria RDTs can give positive results after successful anti-malarial treatment. Therefore, the ***One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag*** *test kit is not recommended for monitoring response to anti-malarial treatment.*
The assay is to be used in the diagnosis of malaria in symptomatic patients as well as asymptomatic patients i.e., pregnant women and children. Testing is not intended for blood donors".

Assay description:

According to the manufacturer's claim, "***One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag*** *test utilizes the principle of immunochromatography in which nitrocellulose membrane is pre-coated with one monoclonal antibody (test line Pf) specific to Histidine-Rich Protein II (Pf-HRP-II) of the Plasmodium falciparum and one monoclonal antibody (test line Pv) specific to lactate dehydrogenase of Plasmodium vivax (Pv-pLDH). Thus, the following Plasmodium antigens are detected in this test:*

- *Histidine Rich Protein II specific for P. falciparum (Pf-HRP-II)*
- *Plasmodium lactate dehydrogenase to Plasmodium vivax (Pv-pLDH)*

The cassette contains a test strip pre-coated with capture antibodies.

The sequence of events is as follows:

1. *Whole blood is applied to the specimen well (labelled well "S").*
2. *Next, buffer is applied to the buffer well (labelled well "B").*
3. *Migration of the blood/buffer mixture starts towards the opposite end of the cassette.*
4. *The blood-buffer mixture passes the conjugate pad, which contains detection antibodies targeting Pf-HRP-II and Pv-pLDH. These detection antibodies are conjugated to colloidal gold. If present in the specimen, Plasmodium target antigens bind to this detection antibody-conjugate.*
5. *The antigen-antibody-conjugate complex migrates further and binds to the capture Plasmodium specific antibodies present on the test line. These capture antibodies bind to another site (epitope) of the Plasmodium target antigens.*
6. *The capture antibodies are applied on a narrow section of the test strip: as a result, the antibody conjugate with the colloidal gold will be concentrated and become visible as a pinkish-purple colored line.*
7. *The excess of the detection antibody-conjugate that was not bound by the Plasmodium target antigens and the capture antibodies moves further to absorbent pad.*
8. *At control zone Goat anti-chicken IgY (as blue coloured line) is immobilized and it binds to IgY colloidal gold conjugate to give a pinkish-purple colored control line. The visualization of the control line indicates* that the migration was successful. It does not confirm the presence of specimen".

Test kit contents

Component	**30 tests/kit (product code MFLRPD-02)**	**25 tests/kit (product code MFLRPD-03)**	**50 tests/kit (product code MFLRPD-04)**	**10 tests/kit (product code MFLRPD-05)**
Cassette packaging, each containing 1 device and 1 desiccant	30	25	50	10
Assay buffer bottles (protein stabilizer, detergent, and preservatives)	2 × 3.0 mL	2 × 3.0 mL	4 x 3.0mL	1 x 3.0mL
Specimen transfer device	30 × 5 µL	25 × 5 µL	50 × 5 µL	10 × 5 µL
Lancet	30	25	50	10
Alcohol swab	30	25	50	10
Pack insert	1	1	1	1

Items required but not provided

- Disposable gloves
- Pen
- Timer
- Sharp box
- Non-sharp disposal container
- Venipuncture blood collection materials and precision pipette plus 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 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, MERISCREEN Malaria Pf /Pv Ag was given priority for WHO prequalification assessment.

Dossier assessment

Meril Diagnostics Pvt. Ltd.submitted a product dossier for **One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag** 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 13 September 2018.

Commitments for prequalification

The evidence of implementation of the commitments for prequalification was accepted by WHO on the 22 January 2020 and the commitments were closed.

Based on the product dossier screening and assessment findings, the product dossier for **One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag** meets WHO prequalification requirements.

Manufacturing site inspection

A desk assessment of **Meril Diagnostics Pvt. Ltd** located at *second floor, D1-D3, Meril Park, Survey No. 135/2/B & 174/2, Muktanand Marg, Chala, Vapi 396191, Gujarat, India* was conducted between 17-19 April 2019. 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 the product of consistent quality. Routine inspections of the Manufacturer will be conducted with copies of these WHO Public Inspection Reports (WHOPIRs) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. To note that a WHOPIR reflects the information on the most current inspection performed at a manufacturing site for in vitro diagnostic products and gives a summary of the inspection findings.

Information on the most current inspection can be found at:

https://www.who.int/diagnostics_laboratory/evaluations/PQDxSiteInspection/en/

All published WHOPIRs are with the agreement of the manufacturer.

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 (78.0% at 200 parasites/µl), *P. vivax* panel detection score (85.7% at 200 parasites/µl), false-positive rates (0.0% for clean negatives, 0.5% for *P. falciparum* at 200 parasites/µl, 0.7% for *P. vivax* at 200 parasites/µl, 0.0% for *P. falciparum* at 2000 to 5000 parasites/µl, x1.4% for *P. vivax* at 2000 to 5000 parasites/µl) and invalid rate (0.0%), One Step test for Malaria *Pf/Pv* Ag MERISCREEN Malaria *Pf/Pv* Ag 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***	***Pf***	***Pv***		
One Step test for Malaria *Pf/Pv* Ag MERISCREEN Malaria Pf/Pv Ag	78	85.7	0.5	0.7	0.0	0.0

Labelling

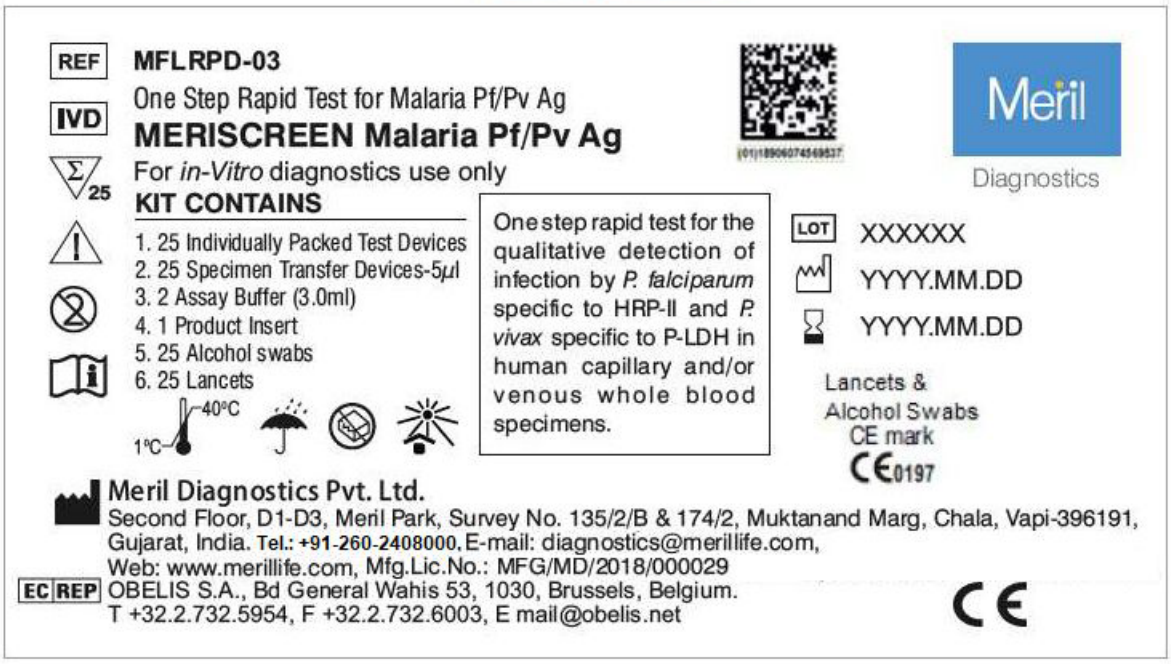
1. **Labels**
2. **Instructions for use**

1. **Labels**

1.1 MERISCREEN Malaria Pf/Pv Ag (MFLRPD-03)- 25 tests configuration

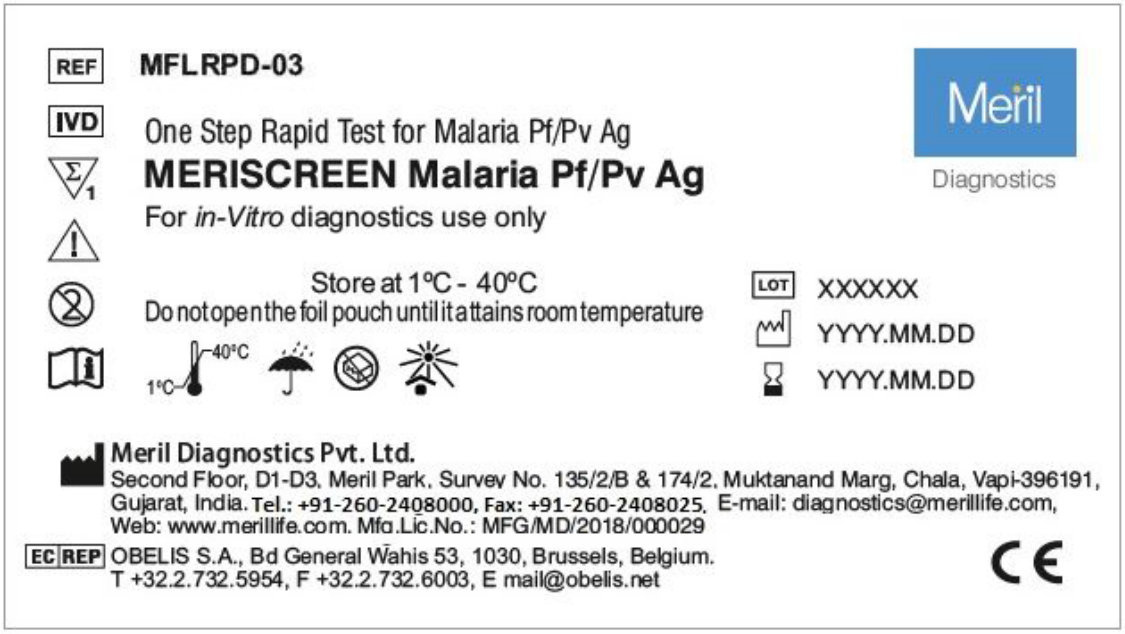
1.1.1 Kit label

Product Box Label



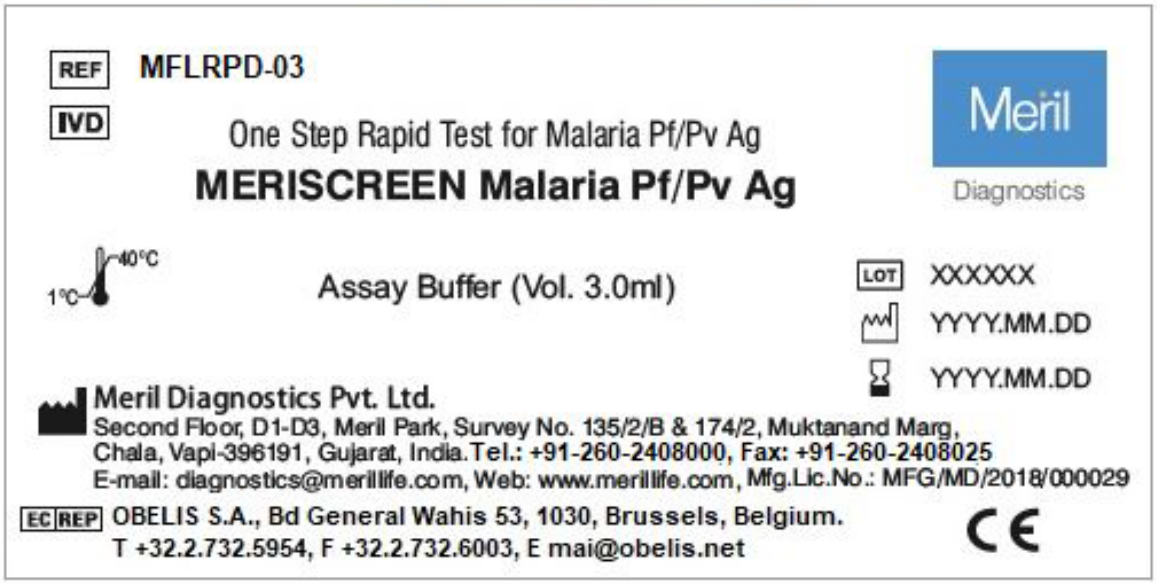
1.1.2 Aluminum pouch label

Aluminum Pouch Label



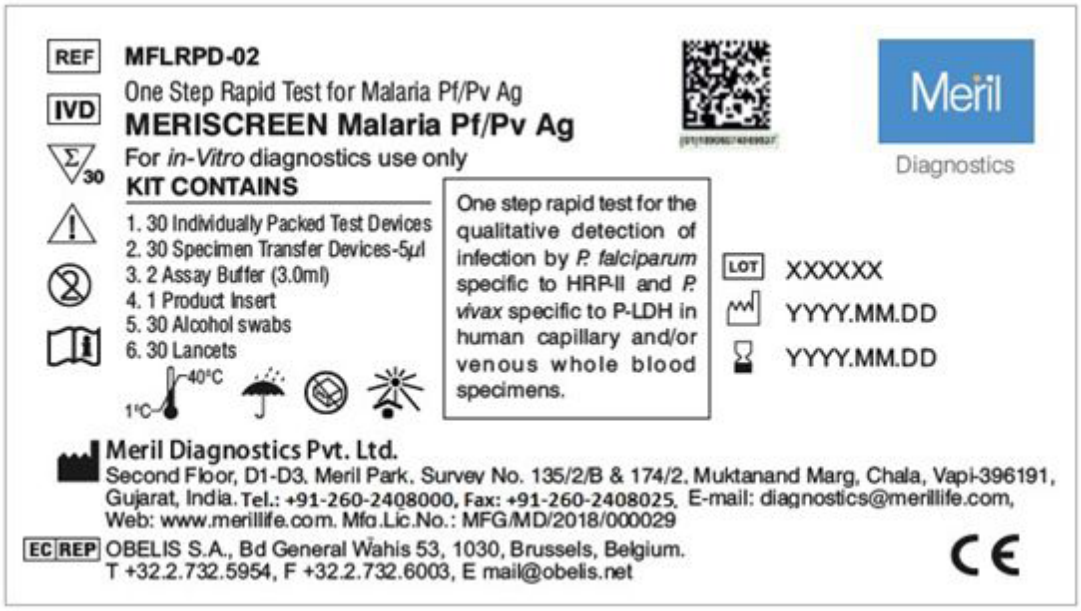
1.1.3 Assay buffer bottle label

Assay Buffer Bottle Label

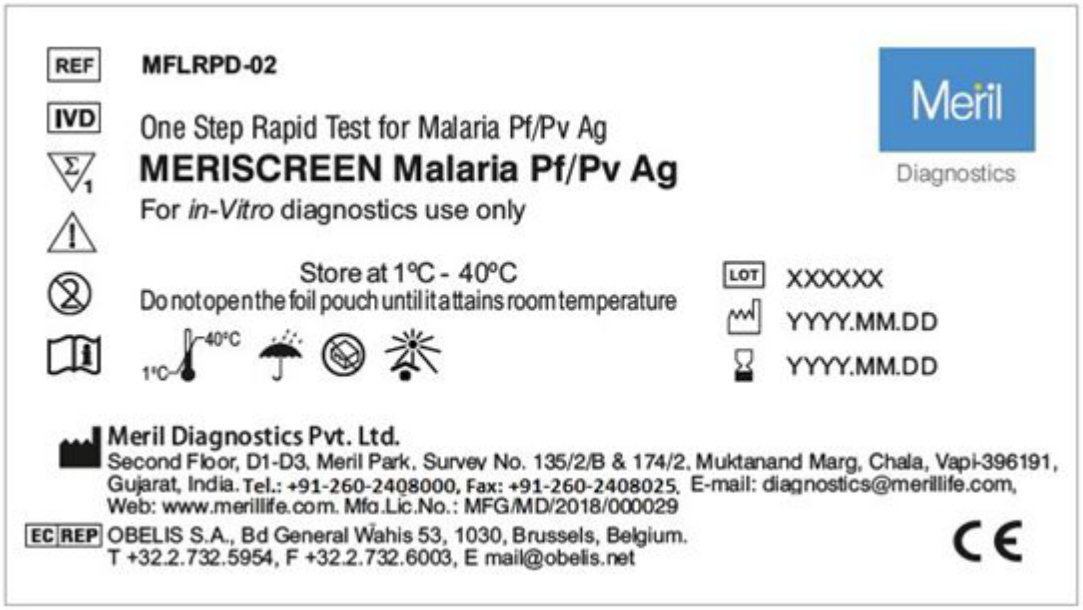


1.2 MERISCREEN Malaria Pf/Pv Ag (MFLRPD-02)-30 tests configuration

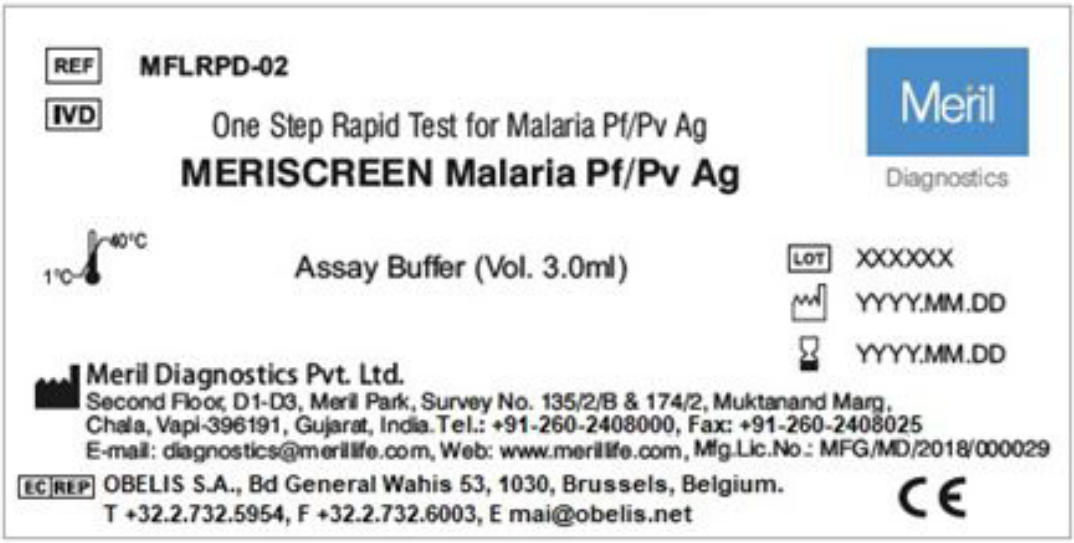
1.2.1 Kit label



1.2.2 Aluminum pouch label

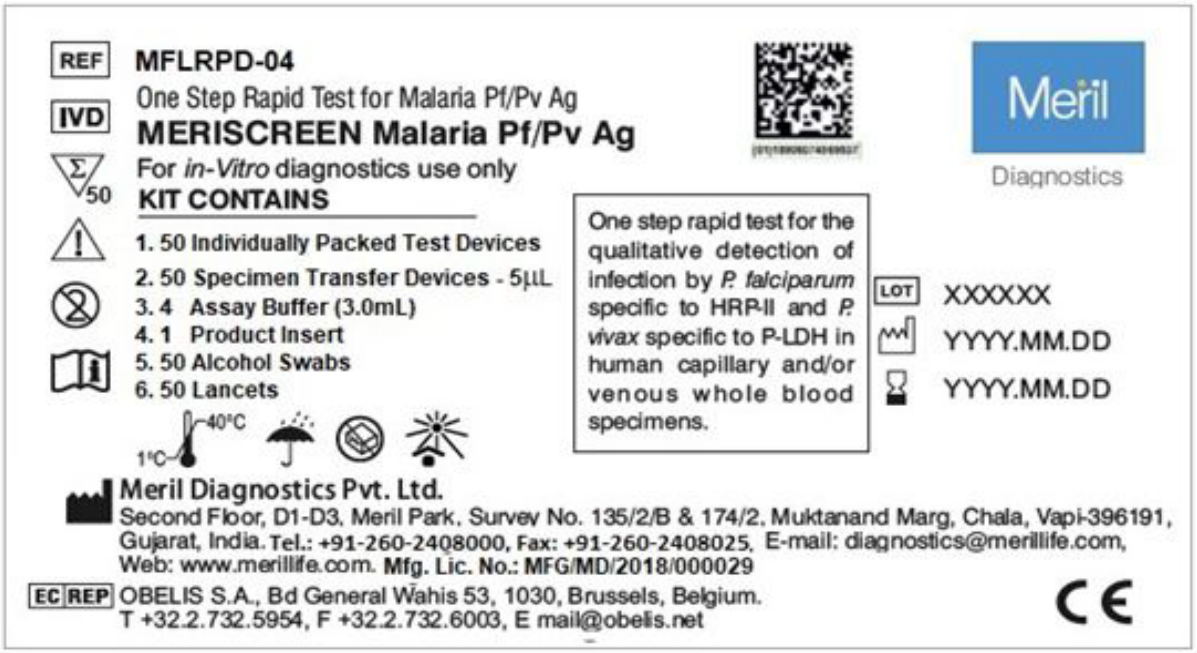


1.2.3 Assay buffer bottle label

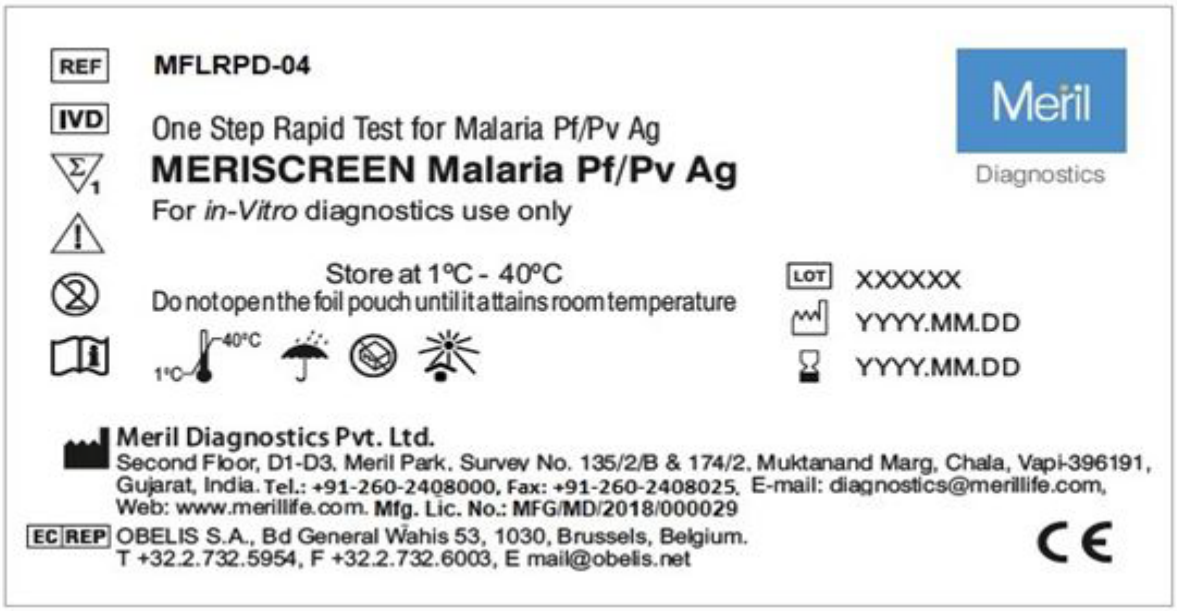


1.3 MERISCREEN Malaria Pf/Pv Ag (MFLRPD-04)-50 tests configuration

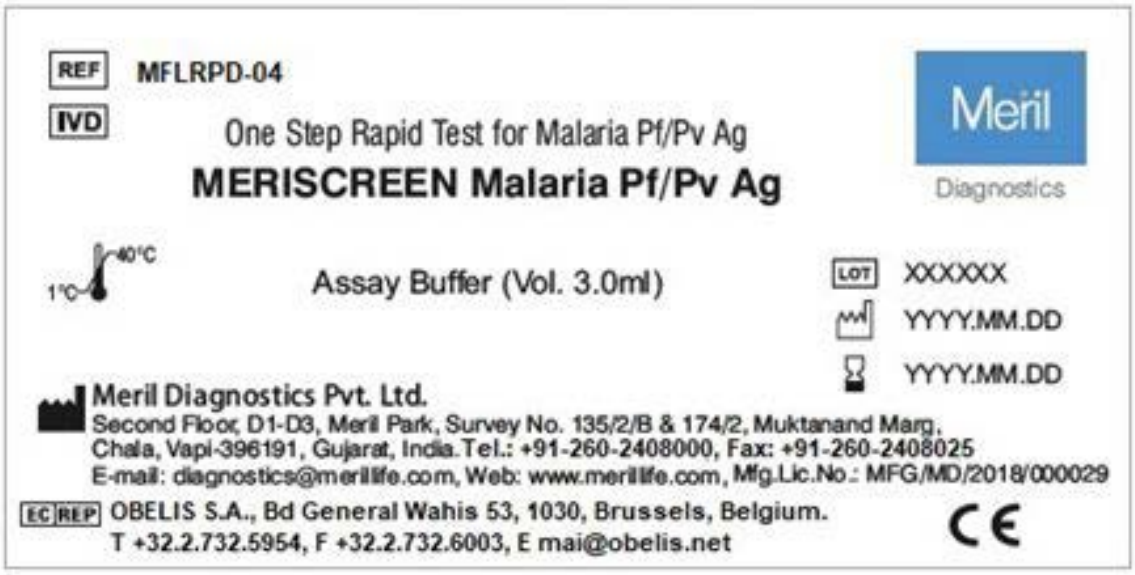
1.3.1 Kit label



1.3.2 Aluminum pouch label



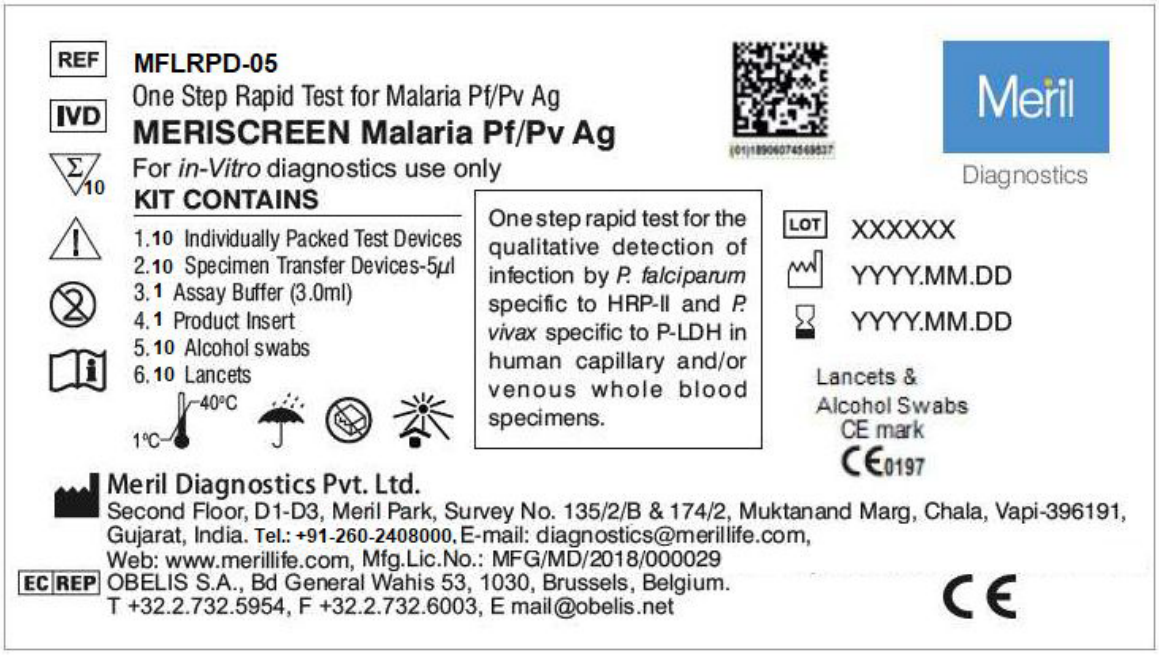
1.3.3 Assay buffer bottle label



1.4 MERISCREEN Malaria Pf/Pv Ag (MFLRPD-05)-10 tests configuration

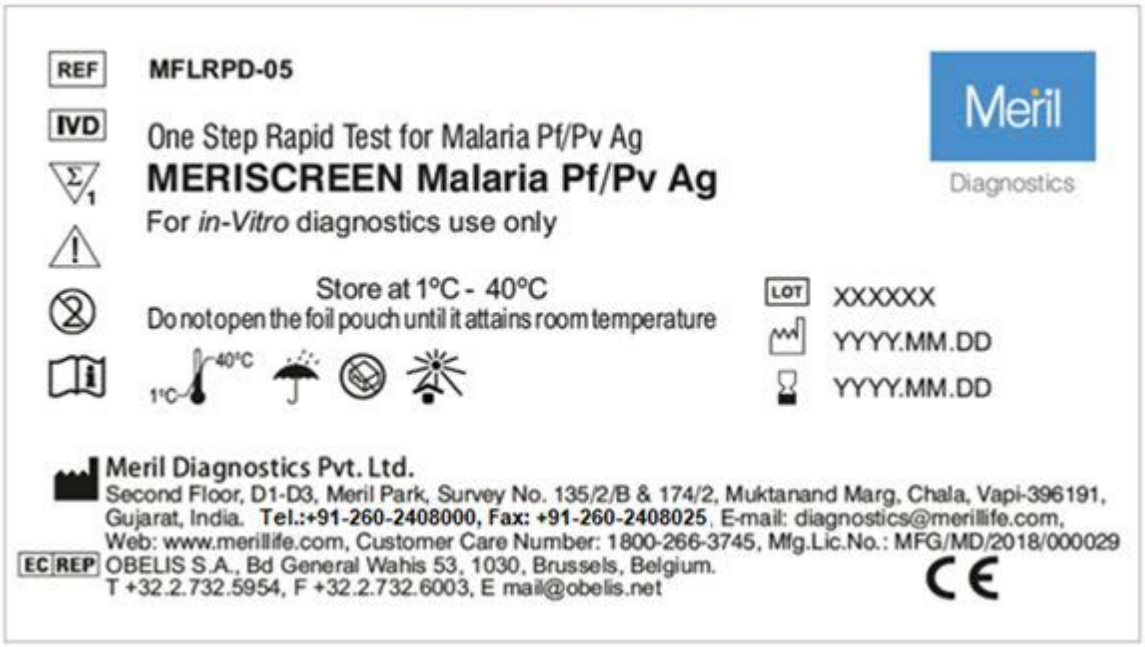
1.4.2 Kit label

Product Box Label



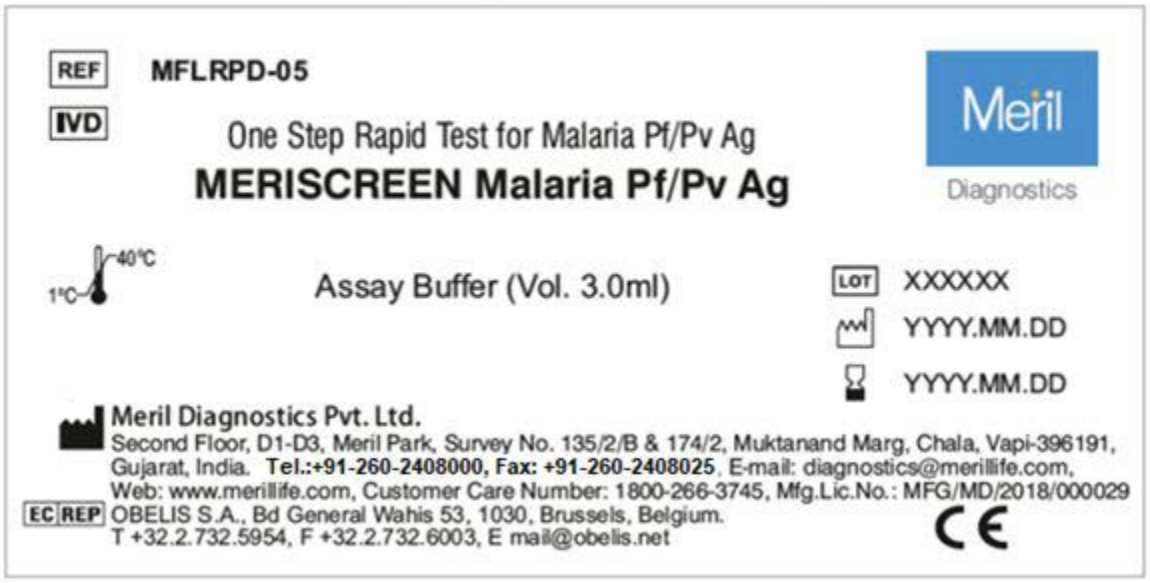
1.4.2 Aluminum pouch label

Aluminum Pouch Label



1.4.3 Assay buffer bottle label

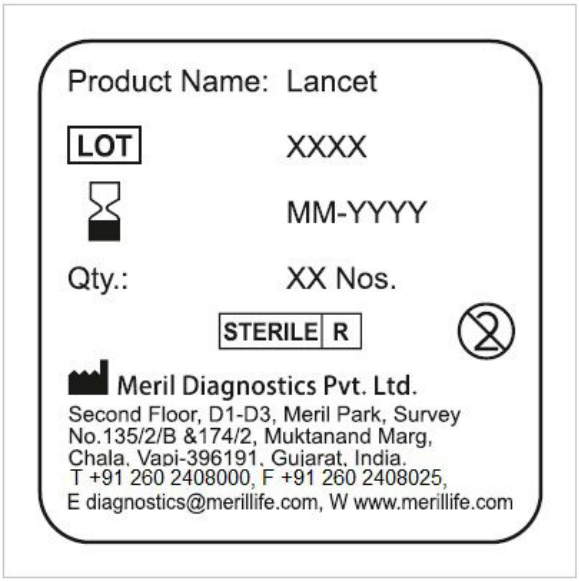
Assay Buffer Bottle Label



1.5 Alcohol swabs label

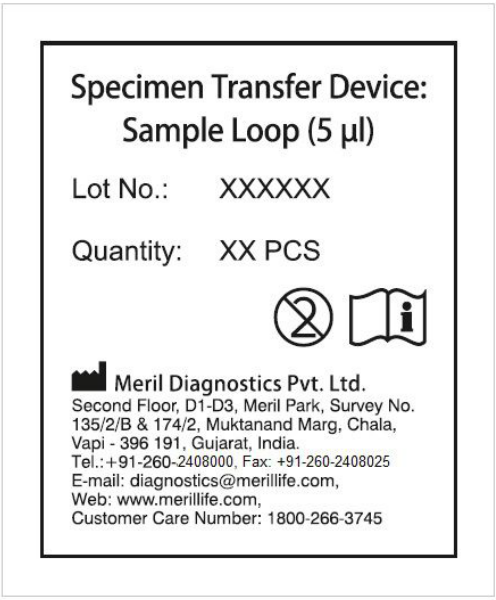
Size: 65mm x 65mm

1.6 Sterile lancet label



Size: 40mm x 40mm

1.7 Specimen transfer device label



Size: 50mm x 60mm

2 Instructions for use[1]

[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.

- Although the test is accurate in detecting HRP-II specific to P. falciparum or pLDH specific to P. vivax in blood specimens, low incidence of false results may occur. Other clinically available tests should be used if questionable results are obtained."Pv" band may turn negative after successful anti-malarial therapy.
- In few cases, HRP-II band appears in certain post treatment malaria, however, such observations are also observed in certain untreated malaria. In such cases, re-testing after 2 days is recommended.
- In *P. falciparum* malaria infection, HRP-II is not secreted in gametogony stage. Hence, in "Carriers", the HRP-II band may be absent.

Note: The presence of the pink-purple control line only means that migration of the test occurred. It does not guarantee that:

- The correct specimen has been used
- The specimen has been applied correctly
- The specimen and test have been correctly stored
- The test procedure was followed correctly

Performance specifications:

A. Sensitivity and Specificity

88 *P. falciparum* positive specimens, 89 *P. vivax* positive specimens including *P. falciparum* & *P. vivax* mixed infection positive specimens, *P. falciparum* and *P. vivax* positive specimens of pregnant women and children were tested with One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag to evaluate the diagnostic sensitivity of One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag test kit. 1000 Plasmodium negative specimens, 107 Plasmodium negative blood donor specimens, 54 Plasmodium negative pregnant women specimens and 66 Plasmodium negative children specimens were tested with One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag to evaluate the Diagnostic Specificity of One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag test kit. The status of the specimen was determined by microscopic examination.

Types of specimens	**% Sensitivity**	**% CI**
Sensitivity of *P. falciparum*	98.86%	95% CI value 93.83% to 99.97%
Sensitivity of *P. vivax*	96.63%	95% CI value 90.46% to 99.30%
Total Sensitivity	97.74%	95% CI value 94.32% to 99.38%
Types of specimens	**% Specificity**	**% CI**
Specificity of *Plasmodium spp*. Negative samples	98.53%	95% CI value 97.69% to 99.13%

B. Analytical Sensitivity (Limit of Detection)

The sensitivity of One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag for *P. falciparum ("Pf" Band)* is ≥50 parasites/µl and for *P. vivax ("Pv" Band) is* ≥200 parasites/µl.

C. Analytical Specificity (Cross reactivity)

To evaluate the interference of One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv 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 One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag test kit is not affected by interfering substances such as, bilirubin (conjugated & unconjugated), triglyceride, acetaminophen, total protein, vitamin B12, sodium azide, thimerosol, alcohol/ethanol, hemoglobin, lipids, aspirin, cross reacting factors such as, Rheumatoid Arthritis, typhoid, pneumonia, diarrhea, filariasis, Hepatitis B, Hepatitis C, syphilis, HIV, dengue and cross reacting antibodies such as, Human Anti-Mouse Antibody (HAMA), Systemic Lupus Erythematous (SLE), Anti-Nuclear Antibodies (ANA).

D. Precision (Repeatability & Reproducibility)

One *P. falciparum* positive of high parasite density i.e., 5634 parasites/µl, one *P. vivax* positive of high parasites density i.e., 6745 parasites/ µl and three Plasmodium negative specimens were utilized for this study. These specimens are further diluted to make moderate and low positive samples. Testing was done by using these samples (high, moderate & low) in replicates of three by three different operators by using three different lots over five (05) days. Total of 1215 tests were generated and 1215 results were obtained. Out 1215, 405 results were generated for Pf, 405 results for Pv and 405 results for Plasmodium negative samples. The results have shown 100% agreement with the sample status when tested with Pf positive, Pv positive and Plasmodium negative samples by three operators, in replicates of three by using three lots over five days. The results & data analysis showed 100% sensitivity for Pf positive and Pv positive samples and 100% specificity for Plasmodium negative samples. The test results have met the acceptance criteria of the study.

Bibliography:

- Carter, R. and Mendis, K.N. (2002). Evolutionary and Historical aspects of the burden of malaria. *Clin. Microbial.,* 15(4):564-594.
- Chandler, J. *et al.,* (2000). The place of gold in rapid tests. *IVD Technology*, 7(2):37-49.
- Moody, A. (2002). Rapid Diagnostics tests for malaria parasites. *Clin. Microbiol*., 15(1): 66-78.
- Murray, C. *et al.,* (2008). Update on Rapid Diagnostics Testing for malaria. *Clin. Microbiol*., 21(1): 97-110.
- Robinson, N. (2002). Immunogold conjugation for IVD applications. *IVD Technology*, 8(3): 33-36.
- Weiss, A. (1999). Concurrent engineering for lateral flow diagnostics. *IVD Technology*, 5(7): 48-57.
- 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.
- Gillet, P. *et al.,* (2011). Prozone in malaria rapid diagnostics tests: how many cases are missed? *Malar J.,* 10:166.
- Jacobs, J. (2014). Harmonization of malaria rapid diagnostic tests: best practices in labelling including instructions for use. *Malar J*, 13:505.
- World Health Organization, Geneva. (2004). Laboratory biosafety manual, third edition.

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/MFLRPD02/04
Date: 28/01/2021

Symbols used on Meril Diagnostics labels:

REF Catalogue No. | Manufacturer | Manufacturing date | Storage temperature | IVD In Vitro Diagnostics | Consult instruction for use

LOT Batch No. | Expiry date | Keep dry | Sufficient for | Caution | EC REP Authorized European Representative in the European Community

For single use only do not reuse | Keep away from direct sunlight | Do not use if box open or damaged | European health & safety product label | CE 0197 This CE mark concerns lancets and alcohol swabs

Manufacturer of Lancets and Alcohol Swabs:
Beijing Ruicheng Medical Supplies Co. Ltd., No. 558 Zhangzikou, Yansong Town, Huairou District, 101400 Beijing, China.
Authorised Representataive of Lancets and Alcohol Swabs:
Lotus Global Co. Ltd., 1 Four Seasons Terrace West Drayton, Middlesex London, UB7 9GG United Kingdom

One Step test for Malaria Pf/Pv Ag

MERISCREEN Malaria Pf/Pv Ag

Product Code	**Pack Size**
MFLRPD-02	30 Test
MFLRPD-03	25 Test
MFLRPD-04	50 Test
MFLRPD-05	10 Test

Intended use:

One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag test kit is an in vitro diagnostic immunochromatographic assay for the qualitative detection of infections with Plasmodium falciparum and P.vivax parasites causing malaria in human whole blood specimens. It does not assess parasite densities.

It assists trained users (in either laboratory or point-of-care settings)

- in detecting Plasmodium falciparum and P.vivax infections
- to differentiate infection between Plasmodium falciparum histidine-rich protein II (Pf-HRP-II) and Plasmodium vivax Plasmodium lactate dehydrogenase (Pv-pLDH)

The assay is intended for trained users and for an initial screening as well as an aid to the diagnosis of malaria infection.

Note: Malaria RDTs can give positive results after successful anti-malarial treatment. Therefore, the One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag test kit is not recommended for monitoring response to anti-malarial treatment.

The assay is to be used in the diagnosis of malaria in symptomatic patients as well as asymptomatic patients i.e., pregnant women and children. Testing is not intended for blood donors.

Principle:

One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag test utilizes the principle of immunochromatography in which nitrocellulose membrane is pre-coated with one monoclonal antibody (test line Pf) specific to Histidine-Rich Protein II (Pf-HRP-II) of the *Plasmodium falciparum* and one monoclonal antibody (test line Pv) specific to lactate dehydrogenase of *Plasmodium vivax* (Pv-pLDH). Thus, the following Plasmodium antigens are detected in this test:

- Histidine Rich Protein II specific for *P. falciparum* (Pf-HRP-II)
- Plasmodium lactate dehydrogenase to *Plasmodium vivax* (Pv-pLDH)

The cassette contains a test strip pre-coated with capture antibodies.

The sequence of events is as follows:

1. Whole blood is applied to the specimen well (labelled well "S").
2. Next, buffer is applied to the buffer well (labelled well "B").
3. Migration of the blood/buffer mixture starts towards the opposite end of the cassette.
4. The blood-buffer mixture passes the conjugate pad, which contains detection antibodies targeting Pf-HRP-II and Pv-pLDH. These detection antibodies are conjugated to colloidal gold. If present in the specimen, Plasmodium target antigens bind to this detection antibody-conjugate.
5. The antigen-antibody-conjugate complex migrates further and binds to the capture Plasmodium specific antibodies present on the test line. These capture antibodies bind to another site (epitope) of the Plasmodium target antigens.
6. The capture antibodies are applied on a narrow section of the test strip: as a result, the antibody conjugate with the colloidal gold will be concentrated and become visible as a pinkish-purple colored line.
7. The excess of the detection antibody-conjugate that was not bound by the Plasmodium target antigens and the capture antibodies moves further to absorbent pad.
8. At control zone Goat anti-chicken IgY (as blue coloured line) is immobilized and it binds to IgY colloidal gold conjugate to give a pinkish-purple colored control line. The visualization of the control line indicates that the migration was successful. It does not confirm the presence of specimen.

The main ingredients of the kit are:

- Test strip
  - Detection antibodies conjugated to colloidal gold :
    - Mouse monoclonal antibodies specific to Pf-HRP-II-gold Colloid
    - Mouse monoclonal antibodies specific to Pv-pLDH-gold Colloid
    - Chicken IgY – gold Colloid
  - Capture antibodies:
    - Test lines
      P.falciparum (Pf) line: Mouse monoclonal antibodies specific to Pf-HRP-II
      P. vivax (Pv) line: Mouse monoclonal antibodies specific to pan-pLDH
    - Control line : Goat anti-Chicken IgY polyclonal antibodies (as blue coloured line)
- Assay buffer
  - Protein stabilizers, detergent and preservatives

Meril Diagnostics

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

Intended User

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

Specimen required:

- Capillary blood or venous blood with the following anticoagulants: EDTA, Heparin, Citrate
- Time between collection and analysis:
  - Capillary: immediately
  - Venous: immediately. If immediate testing is not possible, store the whole blood specimen at 2-8°C for maximum 72 hours (3 days).

Warnings and precautions

- For *in vitro* diagnostic use only.
- Read the instructions carefully before performing the test. The instruction must be followed exactly to get accurate results.
- Apply standard biosafety precautions for handling and disposal of potentially infective material.
  - 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.
- The buffer contains sodium azide as a preservative which may be toxic if ingested. When disposed of through a sink, flush with large quantities of water.
- Do not use any other buffer than the buffer supplied within this kit.
- Do not use the RDT kit beyond the expiration date.
- Do not use if the packaging is damaged.
- Do not use any other specimen than whole blood.
- Do not use if the product has been exposed to excessive heat or humidity.
- Perform the test immediately after opening of the cassette packaging.
- Do not re-use the test.
- Do not touch the nitrocellulose part of the device. Finger print or scratch on nitrocellulose membrane may give erroneous results.
- Do not use the lancet if the seal is broken.
- Do not touch the tip of buffer bottle, it might contaminate buffer.

CE
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, Email: diagnostics@merillife.com, Web: www.merillife.com
EC REP **Obelis s.a., Bd General Wahis 53, 1030, Brussels, Belgium. T +(32) 2 732-59-54, F +(32) 2 732-60-03, E mail@obelis.net**

- Allow all reagents and specimen (s) to attain room temperature (18°C to 30°C) before use.
- Do not smoke, eat or drink while handling specimens and performing a test.
- Contamination of specimen transfer devices and/or reagents can lead to inaccurate results.

Materials

Materials provided

Kit Contents	MFLRPD-02	MFLRPD-03	MFLRPD-04	MFLRPD-05
Cassette packaging, each containing: 1 device 1 desiccant	30	25	50	10
Assay buffer bottle	2 x 3.0 ml	2 x 3.0 ml	4 x 3.0 ml	1 x 3.0 ml
Specimen transfer devices-5 µL	30	25	50	10
Lancets	30	25	50	10
Alcohol Swabs	30	25	50	10
Pack insert	1	1	1	1

Materials required but not provided:

- New pair of disposable gloves
- Pen
- Timer
- Sharp box
- Non-sharps disposal container
- Venipuncture blood collection materials and precision pipette plus 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 excessive 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.

Procedure

Before testing:

- Prepare all necessary materials :
  - When stored in the refrigerator, bring the kit components to room temperature (18°C to 30°C) minimum 30 minutes before use.
  - Prepare all the materials:

Materials Provided	Materials required but not provided
Test Device Assay Buffer bottles Lancets Alcohol swabs Specimen transfer devices Pack Insert	New pair of disposable gloves Pen Timer Sharp box Non-sharps disposal container Venipuncture blood collection materials and precision pipette plus tips (if whole blood is collected by venipuncture)

- Check the expiration date of the test. If expired, do not use it but take another test from an unexpired kit.
- Check that the cassette packaging is not damaged. If damaged, discard the cassette packaging and use another test.
- Open the cassette packaging and check the desiccant. If there is a humidity indicator and it shows saturation (color changed from blue to pink/white), throw away the cassette and take another cassette packaging. If the color of the desiccant does not show any change, you can use the test. Throw away the desiccant in the non-sharps disposal container.
- Take the cassette and place it on a horizontal flat surface.
  You see:
  - a result window (marked with C, Pv, Pf)
  - a circle well marked "S" (for specimen)
  - a square well "B" (for buffer)

- Write the patient name or patient identification number on the cassette.
- Put on gloves. Use new gloves for each patient.

Note: Perform the test immediately after opening of the cassette packaging.
Do not re-use the test.

Test procedure

<u>Capillary whole blood from finger prick</u>

1. Wear gloves.
2. Choose a finger for the finger prick:
   - Do not choose a finger that is swollen, bruised or scarred.
3. Preferably choose the 3rd or 4th finger of the hand which the patient does not use to write.
4. Open the packaging of the alcohol swab. Take out the alcohol swab. Do not throw away the empty packaging (wrapper) but keep it aside.
5. Wipe the complete fingertip with the alcohol swab. Wait until the finger has completely dried (minimum 30 seconds).
6. 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).
7. Take the safety-seal lancet.
8. Detach the cap of the lancet. Puncture the side of the pulp (ball) of the finger with the lancet, perpendicular to the lines of the fingerprint. Dispose the lancet immediately into the sharps box.
9. Make sure a well-formed drop of blood is present on the tip of the finger.
10. If there is no well-formed drop of blood, repeat the finger prick. Use a new lancet and choose a different puncture site.
11. Take the specimen transfer devices and collect 5 µl of blood by dipping the circular end of the specimen transfer devices into the whole blood drop.
12. Place the circular end of the specimen transfer devices in the circle well/specimen well (marked "S") so that it touches the strip (pad at the bottom of the well). Press down lightly to transfer the whole blood to the strip. Put the used specimen transfer devices into the non-sharps disposal container.
13. Take the alcohol swab you put aside (step 5). Ask the patient to press it to the finger prick to stop the bleeding. After use, put the alcohol swab into the non-sharps disposal container.
14. Take the buffer bottle. Hold the open buffer bottle vertically above the square well/buffer well (marked "B"). Squeeze the buffer bottle gently and apply exactly four drops into the square well/buffer well (marked "B").

! Do not use any other buffer than the buffer supplied within this kit.

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

15. Remove your gloves and discard them into the non-sharps disposal container.
16. Write the time on the cassette or set a countdown timer to the required reading time.
17. Read test results after a minimum of 20 minutes but no later than 30 minutes. Use a good light source when reading the test results.

Note: Don't read test results after 30 minutes. Reading too late can give false results.

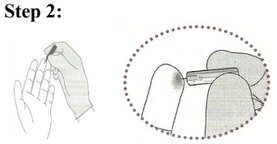
<u>whole blood from venipuncture</u>

1. Wear gloves.
2. Collect blood by standard venipuncture procedure into a tube containing the correct anticoagulant (EDTA, heparin or Citrate).
3. Mix the tube gently.
4. Transfer 5 µl of whole blood using specimen transfer devices in the circle well (marked "S") of the cassette using a precision pipette.
5. Perform steps 12 - 16 of the previous section ("Capillary whole blood from finger prick")

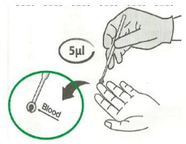
Pictorial Representation of Procedure:

Step 1:

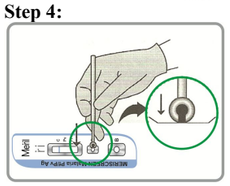
Step 2:



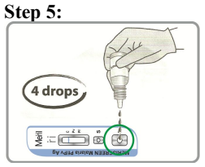
Step 3:



Step 4:



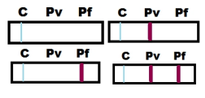
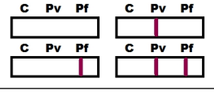
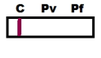
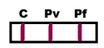
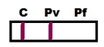
Step 5:



Interpretation of the test result:

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 cassette packaging and repeat the test.
<u>NO</u> Pink Purple line at 'C' (=control)		Invalid. Take a new cassette packaging and repeat the test.
Pink Purple Line at 'C' and **<u>NO</u>** other line		Negative
Pink Purple Line at **'C'** AND at **'Pf'**		Positive for *P. falciparum*
Pink Purple Line at **'C'**, at **'Pf'** AND at **'Pv'**		Positive for *as* a mixed infection with *P. falciparum* and *P. vivax*
Pink Purple Line at **'C'** AND at **'Pv'**		Positive for *P. vivax*

Limitations of the product, causes of false-negative and false-positive results

- The test procedure, precautions and interpretation of result for this test must be followed when testing.
- The test kit is intended for an initial screening as well as an aid to the diagnosis of infection. Other clinically tests are required only if questionable results are obtained. As with all diagnostic tests, the test result must always be co-related with clinical findings.
- The performance of product may be degraded at an ambient temperature of 45°C.
- One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag test kit was tested with interfering substances such as, bilirubin (conjugated & unconjugated), triglyceride, acetaminophen, total protein, vitamin B12, sodium azide, thimerosal, alcohol/ethanol, hemoglobin, lipids, aspirin, cross reacting samples such as, Rheumatoid Arthritis, typhoid, pneumonia, diarrhea, filariasis, Hepatitis B, Hepatitis C, syphilis, HIV, dengue and cross reacting antibodies such as, Human Anti-Mouse Antibody (HAMA), Systemic Lupus Erythematous (SLE), Anti-Nuclear Antibodies (ANA) and the performance of One Step test for Malaria Pf/Pv Ag MERISCREEN Malaria Pf/Pv Ag test kit was not affected by these interfering and cross-reacting factors. Interfering substances, cross reacting factors other than these may affect the performance of the kit.
- False positive result can occur – amongst others in the following conditions:
  - Some viral infection other than hepatitis B or hepatitis C, HIV or dengue
  - Parasitic infection (e.g. Schistosomiasis and Trypanosomiasis)
- Presence of heterophile antibodies in patient's sample other than Rheumatic diseases and autoimmune disorder may lead to false results.
- False negative result can occur in the following conditions:
  - Hook effect due to very high parasite densities i.e., ≥ 26,000 parasites/µl for *P.falciparum* and ≥ 22,000 parasites/µl for *P.vivax*. Repeat the test by using different dilutions of same sample.
  - If antigen concentration/parasite densities present in the specimen is below the detection limits of the assay or the analyte of interest that are detected are not present during the stage of disease in which a sample is collected.
  - deletion in the HRP-II gene resulting in no production of HRP-II antigen
- A positive test should be carefully interpreted to distinguish between new infections and effectively treated old infections. This is due to the persistence of HRP II antigen in the blood for 1-3 weeks after effective treatment. Therefore, malaria RDTs are not recommended for monitoring treatment of malaria.
- This assay cannot be used for the diagnosis of infection by other type of malarial parasites (*P.malariae, P.ovale or P.knowlesi*).
- 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.
- This kit is intended for initial screening of malaria infection as well as an aid to the diagnosis of infection. This test kit can provide fast and easy way to get a result, but do not completely exclude the possibility of false positive or false negative results caused by various factors.