WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: First Response Malaria Ag. pLDH/HRP2 Combo Card Test
WHO reference number: PQDx 0285-010-00

First Response Malaria Ag. pLDH/HRP2 Combo Card Test with product codes **PI16FRC10s, PI16FRC25s, PI16FRC25, PI16FRC30**, **PI16FRC05, and PI16FRC10** manufactured by **Premier Medical Corporation Private Limited**, **rest-of-world regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 04 December 2018.

Summary of WHO prequalification assessment for the First Response Malaria Ag. pLDH/HRP2 Combo Card Test

	Date	Outcome
Prequalification listing	04 December 2018	listed
Dossier assessment	07 September 2018	MR
Site inspection(s) of quality management system	22-24 September 2024	MR
Product performance evaluation	2015	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.

Public report amendment	Summary of amendments	Date of report amendment
Version 2.0	Introducing new suppliers for Alcohol Swab and Twist Lancet resulted in the change of labels.	11 March 2025
Version 3.0	1. Change in the regulatory certification and labelling of the supplier for sterile lancet "Shandong Lianfa Medical Plastic Products Co., Ltd." 2. Change in the label of the alcohol swab supplied by Medtrue Enterprises Co., Limited.	4 June 2025

Version 4.0	Addition of pack sizes of 05 and 10 tests/pack.	12 September 2025

Intended use

According to the claim of the manufacturer "*First Response Malaria Ag. pLDH/HRP2 Combo Card Test is intended to be performed by trained users (in either laboratory or point of care settings) as qualitative screening test for detection of P. falciparum, P. vivax, P. ovale and P. malariae. The test is intended for use with whole blood specimens (capillary or venous blood). Venous blood with the anti-coagulants heparin, EDTA or citrate do not affect the results. This kit is not intended to be used for screening of blood donors. The test is not automated and does not require any additional instrument*."

Assay description

According to the claim of the manufacturer "*First Response Malaria Ag. pLDH/HRP2 Combo Card Test is based on principle of immunochromatography in which nitrocellulose membrane is pre-coated with two monoclonal antibodies as two separate lines. One monoclonal antibody (test line PAN) is PAN-specific to lactate dehydrogenase (pLDH) of the Plasmodium species (Plasmodium falciparum, P. vivax, O. ovale and P. malariae) and the other line (test line P.f.) consists of a monoclonal antibody specific to histidine-rich protein 2 (HRP2) of the P. falciparum. When the test sample along with assay buffer flows through the nitrocellulose membrane, monoclonal antibodies conjugated with colloidal gold, which are pan specific to pLDH and P. falciparum specific to HRP2, binds to Plasmodium antigens released from the lysed blood sample. The antigen-conjugated antibody complex moves through the nitrocellulose membrane and binds to the corresponding immobilized antibody at test lines, which leads to the formation of colour line/lines indicating reactive results. The control line will appear irrespective of reactive or non-reactive sample.*

The First Response Malaria Ag. pLDH/HRP2 Combo Card Test is ''of additional value'' in the differential diagnosis of Plasmodium falciparum and other Plasmodium species."

Test kit contents

<table>
<tr><th>Description</th><th colspan="2">Configuration</th></tr>
<tr><td rowspan="2">Each single test pack contains:
• 1 × Test device & desiccant
• 1 × Specimen Transfer device
• 1 × Alcohol swab
• 1 × Sterile lancet
• 1 × Buffer vial
• 1 × Instructions for use</td><td>10 × single test
(product code PI16FRC10s)</td><td>1 × Master
Instructions for Use</td></tr>
<tr><td>25 × single test
(product code PI16FRC25s)</td><td>1 × Master
Instructions for Use</td></tr>
<tr><td rowspan="4">• Test device with desiccant
• Specimen Transfer device
• Buffer Bottle
• Sterile Lancet
• Alcohol swabs (Optional)
• Instructions for use</td><td>30 × Bulk test
(product code PI16FRC30)</td><td>n/a</td></tr>
<tr><td>25 × Bulk test
(product code PI16FRC25)</td><td>n/a</td></tr>
<tr><td>05 × Bulk test
(product code PI16FRC05)</td><td>n/a</td></tr>
<tr><td>10 × Bulk test
(product code PI16FRC10)</td><td>n/a</td></tr>
</table>

Items required but not provided

- New pair of disposable gloves
- Permanent marker pen
- Timer
- Extra lancets and alcohol swabs, if needed
- Sharp disposable box and biohazardous waste container
- Venipuncture blood collection materials and precision pipette plus tip (if whole blood is collected by venipuncture)
- Bio-hazardous waste container

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.

Prioritization for prequalification
Based on the results of the WHO product testing of malaria RDTs for Round 6, First Response Malaria Ag. pLDH/HRP2 Combo Card Test was given priority for the WHO prequalification assessment.

Dossier assessment

Premier Medical Corporation Private Limited submitted a product dossier for **First Response Malaria Ag. pLDH/HRP2 Combo Card 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 07 September 2018.

Based on the product dossier screening and assessment findings, the product dossier for **First Response Malaria Ag. pLDH/HRP2 Combo Card Test** meets WHO prequalification requirements.

Manufacturing site inspection

An onsite inspection of Premier Medical Corporation Private Limited., at A1-302 and 3704-05, GIDC, Sarigam INA, 396155 Gujarat, India, was conducted from the 22nd to 24th of September 2024. 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 assessment performed at a manufacturing site for in vitro diagnostic products and summarizes the assessment findings.

https://extranet.who.int/pqweb/vitro-diagnostics/who-public-inspection-reports

All published WHOPIRs are with the agreement of the manufacturer.

The onsite inspection was accepted on 17 February 2025.

Based on the site inspection and corrective action plan review, the quality management system for First Response Malaria Ag. pLDH/HRP2 Combo Card Test meets WHO prequalification requirements.

Product performance evaluation

The sixth round of WHO product testing of RDTs for malaria antigen detection was completed in 2015. 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 (82% at 200 parasites/µl), *P. vivax* panel detection score (91.4% at 200 parasites/µl), false-positive rates (1.9% for clean negatives, 1.5% for *P. falciparum* at 200 parasites/µl, 0.0% for *P. vivax* at 200 parasites/µl, 0.0% for *P. falciparum* at 2000 to 5000 parasites/µl, 0.0% for *P. vivax* at 2000 to 5000 parasites/µl) and invalid rate (0.1%), **First Response Malaria Ag. pLDH/HRP2 Combo Card 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	Pf	Pv		
First Response Malaria Ag. pLDH/HRP2 Combo Card Test	82.0	91.4	1.5	0.0	1.9	0.1

Labelling

1. **Labels**
2. **Instructions for Use**

1. Labels

Alcohol swab labels

MEDTRUE®

Alcohol Swabs

CE

CODE:MT59010102

LOT LOT NO. : 202412

MFG. DATE: 20241201

EXP. DATE : 20291130

For External Use Only

CONTAIN:One pad saturated with 70% Isopropyl Alcohol.

DIRECTION:Cleaning the required area.

Discard after single use.

MEDTRUE ENTERPRISE CO., LTD
Room No.301-302, Hongpujiezuo Mansion
186-1 Jiangdongzhonglu Road, 210019 Nanjing, China

CE EC REP RIOMAVIX SOCIEDAD LIMITADA
Calle de Almansa 55,1D,Madrid 28039 Spain

TEAR HERE

PHX2006-NS

Phoenix
Healthcare Solutions, LLC
"An Innovative Medical Manufacturer"

Alcohol Prep Pad

70% v/v Isopropyl Alcohol

For External Use Only

1 Pad

Medium

Discard Prep Pad After Single Use

Directions:
Apply topically as needed to cleanse intended area

Phoenix Innovative Healthcare Manufacturers Pvt. Ltd.
EL-209, Shil Mahape Road, Electronic Zone,
MIDC, TTC Industrial Area, Mahape,
Navi Mumbai - 400 710 MH | India
Customer Care : 022-61075501
Email : customercare@phoenix-hs.com
NCE-MH/DRUGS/25-MH/101592

EC REP
Advena Ltd.,
Tower Business Centre,
2nd Flr, Tower Street,
Swatar, BKR 4013. Malta

CE

MD

NON STERILE

LOT XXXXXXXX

YYYY-MM

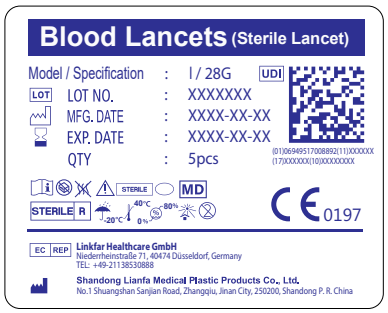
YYYY-MM

Rev.00

Sterile Twist lancet

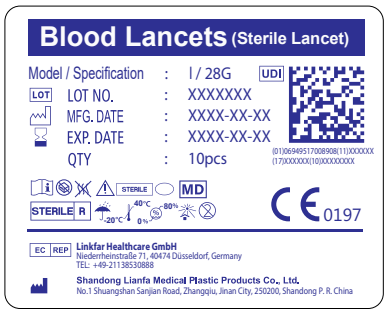
PANTONE Reflex Blue C

Size:50*40mm



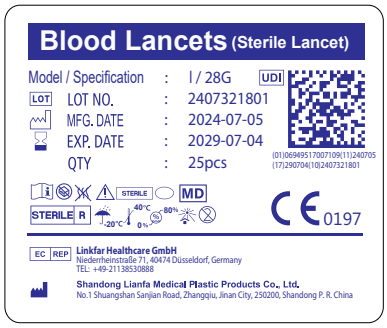
PANTONE Reflex Blue C

Size:50*40mm



PANTONE Reflex Blue C

Size:50*42mm



PANTONE Reflex Blue C

Size:50*42mm

Blood Lancets (Sterile Lancet)

Model / Specification : I / 28G

LOT LOT NO. : 2407321802

MFG. DATE : 2024-07-12

EXP DATE : 2029-07-11

QTY : 30pcs

UDI

(01)06949517007116(11)240712
(17)290711(10)2407321802

STERILE MD

STERILE R

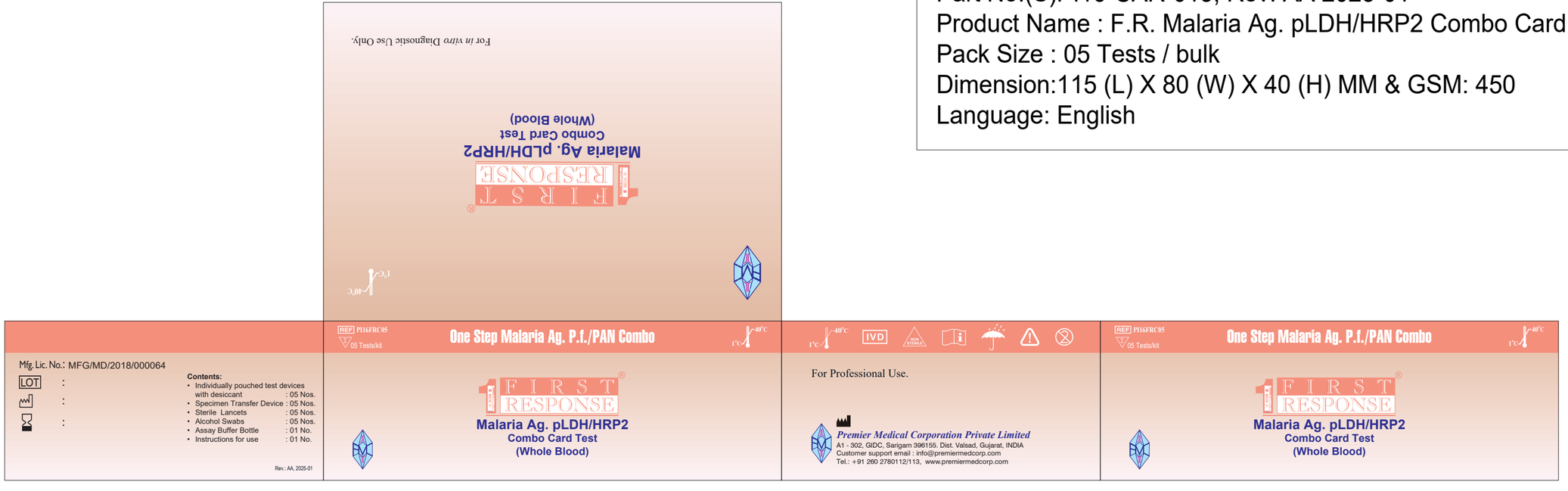
-20°C 40°C 0% 80%

CE 0197

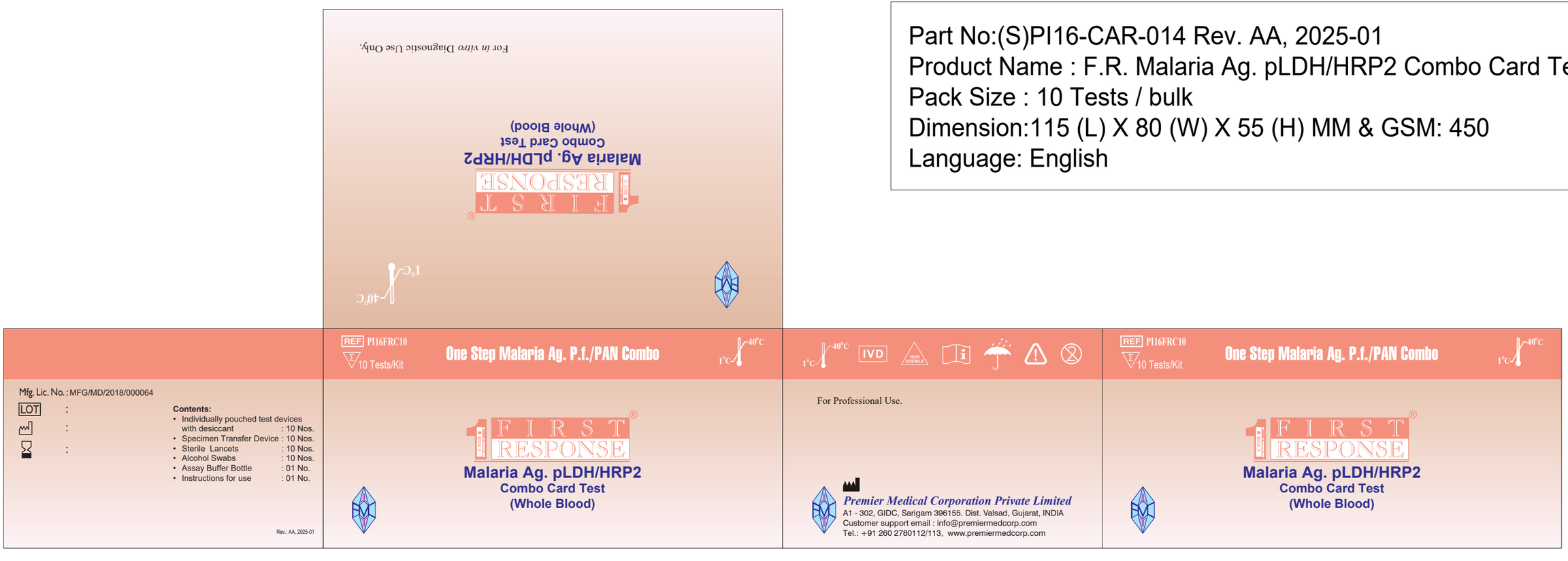
EC REP **Linkfar Healthcare GmbH**
Niederrheinstraße 71, 40474 Düsseldorf, Germany
TEL: +49-21138530888

Shandong Lianfa Medical Plastic Products Co., Ltd.
No.1 Shuangshan Sanjian Road, Zhangqiu, Jinan City, 250200, Shandong P. R. China

Part No:(S)PI16-CAR-013, Rev. AA 2025-01
Product Name : F.R. Malaria Ag. pLDH/HRP2 Combo Card Test
Pack Size : 05 Tests / bulk
Dimension:115 (L) X 80 (W) X 40 (H) MM & GSM: 450
Language: English



Part No:(S)PI16-CAR-014 Rev. AA, 2025-01
Product Name : F.R. Malaria Ag. pLDH/HRP2 Combo Card Test
Pack Size : 10 Tests / bulk
Dimension:115 (L) X 80 (W) X 55 (H) MM & GSM: 450
Language: English



Product Name : FR Malaria Ag.*pLDH/HRP2* Combo Card Test
Pack Size : 25 Tests / bulk

For *in vitro* Diagnostic Use Only.

Malaria Ag. pLDH/HRP2
Combo Card Test
(Whole Blood)

FIRST RESPONSE

1°C 40°C

REF PI16FRC25 Σ 25 Tests/kit **One Step Malaria Ag. P.f. / Pan Combo** 1°C 40°C

Mfg. Lic. No.: MFG/MD/2018/000064

LOT :

:

:

Contents:

• Individually pouched test devices with desiccant	: 25 Nos.
• Specimen Transfer Device	: 25 Nos.
• Sterile Lancets	: 25 Nos.
• Alcohol Swabs	: 25 Nos.
• Assay Buffer Bottle	: 1 No.
• Instructions for use	: 1 No.

Rev.: AD, 2020-10

FIRST RESPONSE

Malaria Ag. pLDH/HRP2
Combo Card Test
(Whole Blood)

1°C 40°C IVD NON STERILE

For Professional Use.

Premier Medical Corporation Private Limited
A1 - 302, GIDC, Sarigam 396155. Dist. Valsad, Gujarat, INDIA
Customer support email : info@premiermedcorp.com
Tel.: +91 260 2780112/113, www.premiermedcorp.com

REF PI16FRC25 Σ 25 Tests/kit **One Step Malaria Ag. P.f. / Pan Combo** 1°C 40°C

FIRST RESPONSE

Malaria Ag. pLDH/HRP2
Combo Card Test
(Whole Blood)

Product Name : FR Malaria Ag.*pLDH/HRP2* Combo Card Test
Pack Size : 30 Tests / bulk

For *in vitro* Diagnostic Use Only.

Malaria Ag. pLDH/HRP2
Combo Card Test
(Whole Blood)

FIRST RESPONSE

1°C 40°C

Mfg. Lic. No. : MFG/MD/2018/000064

LOT :

:

:

Contents:

• Individually pouched test device with desiccant	: 30 Nos.
• Specimen transfer device	: 30 Nos.
• Sterile Lancet	: 30 Nos.
• Alcohol Swab	: 30 Nos.
• Assay buffer bottle	: 1 No.
• Instructions for use	: 1 No.

Rev.: AB, 2024-10

REF PI16FRC30
Σ 30 Tests/kit

One Step Malaria Ag. P.f. / Pan Combo

1°C 40°C

FIRST RESPONSE

Malaria Ag. pLDH/HRP2
Combo Card Test
(Whole Blood)

1°C 40°C IVD NON STERILE

For Professional Use

Premier Medical Corporation Private Limited
A1 - 302, GIDC, Sarigam 396155. Dist. Valsad, Gujarat, INDIA
Customer support email : info@premiermedcorp.com
Tel.: +91 260 2780112/113, www.premiermedcorp.com

REF PI16FRC25
Σ 30 Tests/kit

One Step Malaria Ag. P.f. / Pan Combo

1°C 40°C

FIRST RESPONSE

Malaria Ag. pLDH/HRP2
Combo Card Test
(Whole Blood)

Product Name : FR Malaria Ag. pLDH/HRP2 Combo Card Test
Pack Size : 25 Single Tests

Mfg. Lic. No. : MFG/MD/2018/000064

LOT :

:

:

Materials Provided :

- 25 Single Test Pack
  Each single test pack contents:
  (Test device & desiccant, Specimen transfer device, Alcohol swab, Sterile lancet, Buffer vial & Instructions for use.)
- Master Instructions for use: 1 No.

Rev.: AD, 2022-05

REF PI16FRC25s
Σ 25 Tests/kit

One Step Malaria Ag. P.f. / Pan Combo

1°C 40°C

FIRST RESPONSE®

Malaria Ag. pLDH/HRP2
Combo Card Test
(Whole Blood)

1°C 40°C IVD NON STERILE

For Professional Use.

Premier Medical Corporation Private Limited
A1 - 302, GIDC, Sarigam 396155. Dist. Valsad, Gujarat, INDIA
Customer support email : info@premiermedcorp.com
Tel.: +91 260 2780112/113, www.premiermedcorp.com

REF PI16FRC25s
Σ 25 Tests/kit

One Step Malaria Ag. P.f. / Pan Combo

1°C 40°C

FIRST RESPONSE®

Malaria Ag. pLDH/HRP2
Combo Card Test
(Whole Blood)

FIRST RESPONSE®

Malaria Ag. pLDH/HRP2
Combo Card Test
(Whole Blood)

1°C 40°C

For *in vitro* Diagnostic Use Only.

For *in vitro* Diagnostic Use Only.

Malaria Ag. pLDH/HRP2
Combo Card Test
(Whole Blood)

FIRST RESPONSE

1°C 40°C

Product Name : FR Malaria Ag.pLDH/HRP2 Combo Card Test
Pack Size : 10 Single Tests

REF PI16FRC10s
Σ 10 Tests/kit
One Step Malaria Ag. P.f. / Pan Combo
1°C 40°C

Mfg. Lic. No.: MFG/MD/2018/000064

LOT :

:

:

Material Provided:

- 10 Single Test Pack
  Each single test pack contents:
  (Test device & desiccant, Specimen Transfer Device, Alcohol swab, Sterile lancet, Buffer Vial & Instructions for use.)
- Master Instruction for use : 1 No.

Rev.: AB, 2024-10

FIRST RESPONSE®

Malaria Ag. pLDH/HRP2
Combo Card Test
(Whole Blood)

1°C 40°C IVD NON STERILE

For Professional Use.

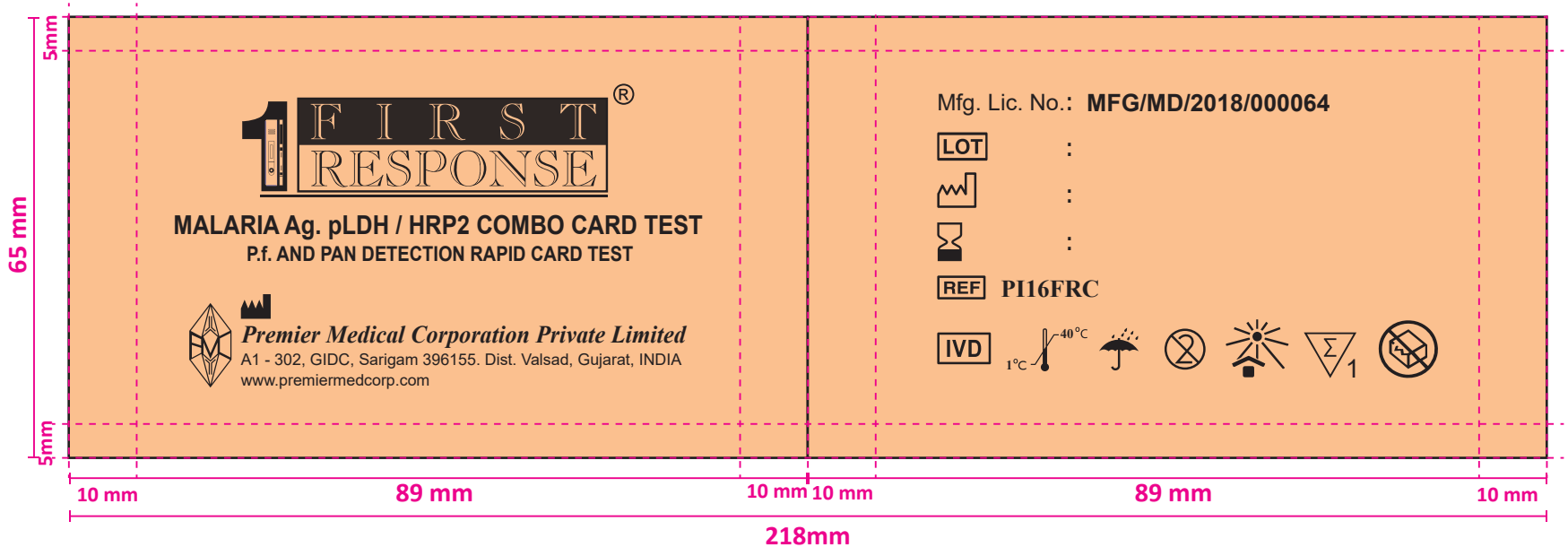
Premier Medical Corporation Private Limited
A1 - 302, GIDC, Sarigam 396155. Dist. Valsad, Gujarat, INDIA
Customer support email : info@premiermedcorp.com
Tel.: +91 260 2780112/113, www.premiermedcorp.com

REF PI16FRC10s
Σ 10 Tests/kit
One Step Malaria Ag. P.f. / Pan Combo
1°C 40°C

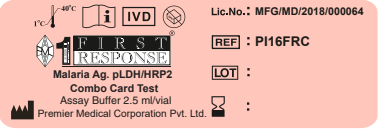
FIRST RESPONSE®

Malaria Ag. pLDH/HRP2
Combo Card Test
(Whole Blood)

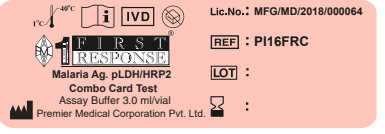
Product Name : Aluminum pouch - F.R Malaria Ag.pLDH/HRP2 Combo Card Test



Assay buffer label- First Response Malaria Ag. pLDH/HRP2 Combo Card Test (2.5 ml)



Assay buffer label- First Response Malaria Ag. pLDH/HRP2 Combo Card Test (3.0 ml)



2. Instructions for use[1]

Instructions for Use- Bulk pack size

[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.

14) Do not eat the dessicant.
15) Do not use any other specimen other than human whole blood. Do not mix and interchange different specimens.

Specimen collection and storage

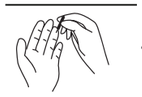
[Collection by venipuncture]

1) Collect the whole blood into the collection tube (containing EDTA/sodium citrate/heparin) by venipuncture.
2) If specimens are not immediately tested (within 1 hour) should be stored at 2-8 °C maximum upto 72 hours (3 days).Using the specimen more than three days can cause non-specific reaction.

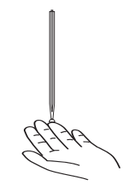
Capillary blood specimen collection:

- Wear gloves, massaging the fingertip gently. It will help to obtain a round drop of blood.

- Wipe the complete finger tip with the alcohol swab. Wait until the finger tip dried completely.
- Detach the protective cap of the lancet and pierce the end of finger tip with the sterile lancet provided.



- Gently squeeze the area until you get enough blood specimen.



- After completion of specimen collection, take the used alcohol swab of same patient and press it on the finger to stop the bleeding.

  Note : The lancet should only be used once. Dispose of used lancets in sharp box and used alcohol swab in biohazardous waste container.

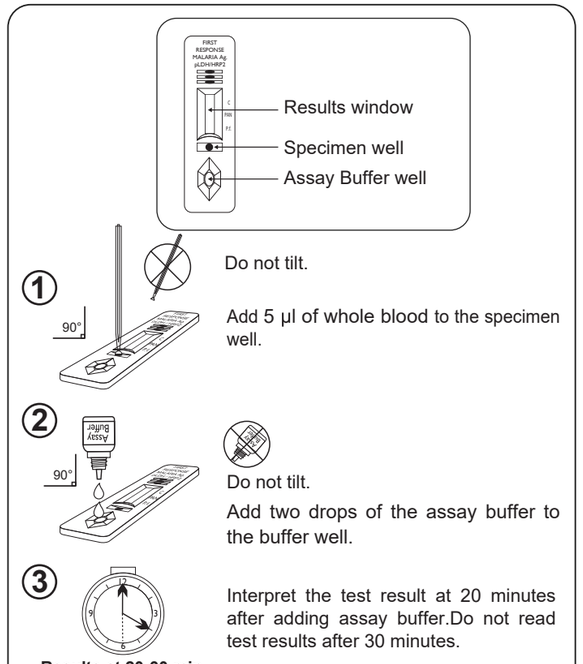
- Do not share used lancets with another person. To prevent possible infection, a used lancet should not be touched by another person.
- Do not use expired lancet. The use of an expired lancet may cause any infection at the punctured skin due to cease to exist its sterility.
- Use new lancet and choose a different puncture site, if repeat the finger prick.
- Do not share used alcohol swab.

Specimen storage

1) Whole blood specimen may be used for testing immediately (within 1 hour) or may be stored at 2-8°C for maximum up to 72 hours (3 days). Do not use blood specimen stored for more than 3 days, It can cause non-specific reaction.

Test Procedure

1) Bring the First Response® Malaria Ag. pLDH/HRP2 Combo Card Test kit components to room temperature (15 - 40°C) prior to 15 minutes of testing.
2) Remove the Test Device and the Specimen transfer Device from the kit and place it on a flat, dry surface and label the Test Device with specimen identification number/name.
3) Slowly add 5 µl of whole blood to the specimen well using the Specimen Transfer Device. Dispose the used Specimen transfer device as biohazard waste immediately after use.
4) Add two drops of the Assay buffer to the buffer well.
5) Observe for development of colored bands in the Results Window.
6) Interpret test results at 20 minutes. (After recording the results, dispose of test device as a biohazardous waste).
7) Do not interpret after 30 minutes.



Caution

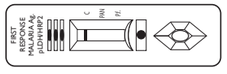
- Hold specimen transfer device and assay buffer bottle vertically, else it can lead to inaccurate results.
- Exactly 2 drops of assay buffer should be added. Adding more than or less than 2 drops may cause over flooding or reverse migration phenomenon, which may lead inaccurate results of the test.
- Results can be interpreted any time from 20 to 30 minutes.Do not read test result after 30 minutes. Reading beyond 30 minutes may give inaccurate results. After recording the results, dispose of test device as a biohazard waste.

Internal Quality Control

The visualization of the control line in First Response® Malaria Ag. pLDH/HRP2 Combo Card Test indicates that active ingredient of the strips are functional and the migration is successful. The control line in First Response® Malaria Ag. pLDH/HRP2 Combo Card Test is not meant for specimen addition monitoring.

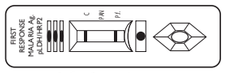
How to Interpret test results

Negative Results



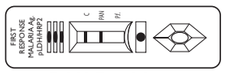
If only one color line appear, at control line 'C' as in the figure, the specimen is negative.

Positive Results



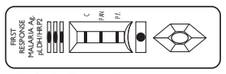
***P.f.* Positive**

If two color lines appears, one at control line 'C' and other at test line P.f. as in the figure, the specimen is reactive for antigens to P.f. Interpret faint line as reactive line.



PAN Positive

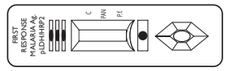
If two color lines appears, one at control line 'C' and other at test line PAN as in the figure, the specimen is reactive for antigens to PAN. Interpret faint line as reactive line.



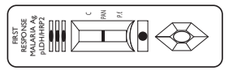
***P.f.* & PAN Positive**

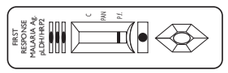
If all three color lines appears, one at control line 'C' and other two at test lines P.f. and PAN as in the figure, the specimen is reactive for antigens to P.f. and PAN. Interpret faint line as reactive line.

Invalid Results

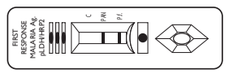


No presence of control line 'C' in the result window (irrespective of presence of test lines) indicates an invalid result.





The directions may not have been followed correctly or the test may have deteriorated.



The Invalid test results should be retested with new test device.

Performance Characteristics

First Response® Malaria Ag. pLDH/HRP2 Combo Card Test were tested using an in-house panel of Positive and Negative clinical specimens characterized by malaria microscopy as the reference method. First Response® Malaria Ag. pLDH/HRP2 Combo Card Test showed 100% sensitivity and 100% specificity. First Response® Malaria Ag. pLDH/HRP2 Combo Card Test showed 100% agreement with the reference method.

Specimen details	Reference Method (Microscopy)		First Response® Malaria Ag. pLDH/HRP2 Combo Card Test		
	Positive	Negative	Positive	Negative	Total
P. falciparum Positive Whole blood specimen	231	0	231	0	231
P. vivax Positive Whole blood specimen	243	0	243	0	243
Malaria Negative Whole blood specimen	0	1287	0	1287	1287
Total	474	1287	474	1287	1761

Reference Method	Specimen details		First Response® Malaria Ag. pLDH/HRP2 Combo Card Test			
	Clinical Status	Parameter	Positive	Negative	Total Result	95% Confidence Interval
Microscopy	*P. falciparum* Positive	Sensitivity	231	00	231	(97.96%-100%)
	P. Vivax Positive	Sensitivity	243	00	243	(98.06%-100%)
	Malaria Negative	Specificity	00	1287	1287	(99.63%-100%)

Note: The product was not been fully assessed with P.ovale & P. malariae and the sensitivity is expected to be low (<50 %)

Worldwide Performance Panel

The analytical sensitivity of the First Response® Malaria Ag. pLDH/HRP2 Combo Card Test was carried out by testing WHO worldwide performance panel. Total 10 specimens were tested in-house. The First Response® Malaria Ag.pLDH/HRP2 Combo Card Test showed 100% Sensitivity.

Analytical Sensitivity : In-House Evaluation			
Total Specimens		First Response® Malaria Ag.pLDH/HRP2 Combo Card Test	
		Positive	Negative
05	200 p/µl	05	00
05	2000 p/µl	05	00

Cross Reactivity Study

First Response® Malaria Ag. pLDH/HRP2 Combo Card Test was tested with specimen reactive for other diseases/conditions (mentioned in following table), which may interfere with performance of the test. The First Response® Malaria Ag. pLDH/HRP2 Combo Card Test tested with mentioned specimen for cross reactivity study as well as same diseased/condition specimens# were also used for spiking of malaria positive specimens# to determine effect on sensitivity of the test. None of the specimens interfere with the test results of First Response® Malaria Ag.pLDH/HRP2 Combo Card Test, and showed no cross reactivity with 100% sensitivity.

Specimens tested	*P. falciparum* positive specimens	*P. vivax* positive specimens	Malaria negative specimens	Specimens tested	*P. falciparum* positive specimens	*P. vivax* positive specimens	Malaria negative specimens
Syphilis Positive#	06	06	10	HSV 1/2 Positive#	05	05	05
HIV Positive#	03	03	05	HTLV- I Ab Positive#	07	07	07
Dengue NS1 Positive#	05	05	05	HTLV- II Ab Positive#	09	09	09
Multipara (Pregnant Woman)	08	04	72	HSV - I IgG Positive#	08	08	08
CMV Positive#	03	03	03	Rubella IgG & IgM Positive#	15	15	15
ANA Positive#	04	04	04	HBsAg Positive#	03	03	05
HAV Positive#	04	04	04	Chikungunya Positive#	05	05	05
EBV Positive#	02	02	02	Anti-malarial drug medication	02	02	Not tested
HCV Positive#	03	03	05	Anti-TB drug medication#	03	03	03
Yellow fever virus# post immunization	03	03	04	Infuenza A and B#	03	03	06
Measles#	03	03	04	Visceral leishmaniasis#	03	03	05
Leptospirosis#	03	03	05	Bilirubin#	03	03	05
Cholesterol# Triglycerides	03	03	05	Chagas	03	03	05
Influenza vaccine# recipient	03	03	05	Sickle cell	03	03	05
Leishmaniasis#	03	03	05	Acute hepatitis A infection#	03	03	05
Schistosomiasis#	03	03	05	Toxoplasmosis#	03	03	05

Potential interference substances

The interfering substances that may affect performance of the First Response® Malaria Ag.pLDH/HRP2 Combo Card Test are mentioned in following table. The First Response® Malaria Ag.pLDH/HRP2 Combo Card Test showed no reactivity with any of mentioned specimens and showed 100% specificity. The same specimens# were spiked in malaria positive specimens respectively and tested.First Response® Malaria Ag.pLDH/HRP2 Combo Card Test showed 100% sensitivity with spiked specimens.

Specimens tested	*P. falciparum* positive specimens	*P. vivax* positive specimens	Malaria negative specimens	Specimens tested	*P. falciparum* positive specimens	*P. vivax* positive specimens	Malaria negative specimens
Lipemic specimen#	05	05	05	Low Hematocrit specimens	Not tested	Not tested	05
Icteric specimens#	05	05	05	Whole blood specimen in ACD anticoagulant	03	03	182
Hemolytic specimens	01	01	05	RF Ab Positive#	04	04	09
High Hematocrit specimens	Not tested	Not tested	05	dsDNA Antibody Positive#	01	01	01
Recipient of multiple blood transfusion	03	03	05				

Precision

a) Within run, precision was determined by using 225 replicates of 9 different specimens containing different malaria parasitic count. Within run, precision was observed 100%.
b) Between run, precision was determined by using the 9 different specimens containing different malaria parasitic count in 5 different replicates with 3 different lots of test devices X 5 different days X 3 different sites tested.
Between run, precision was observed 100%.

External Evaluation Report

Place of Evaluation	Year	Sensitivity				Specificity	
		PAN		P.f.		PAN	P.f.
		200 p/µl	2000 p/µl	200 p/µl	2000 p/µl		
WHO Evaluation Round 6	2014 - 2015	91.4%	100%	82%	100%	100%	100%
Ministry of Health & Children Care Zimbabwe	2014	92.5%		95.5%		98.78%	98.38%
National Public Health & Reference Lab, Ghana	2015	96%		100%		100%	100%

Potential interference drug substances

The details of interference drug molecules are mentioned in the following table. Each interfering drug molecule substance was spiked at the final concentration of 250µg/ml in malaria positive specimens as well as negative specimens, respectively. No false positive or false negative results were observed with any drug molecules, when tested with First Response® Malaria Ag. pLDH/HRP2 Combo Card Test.

Diclofenac	Acetaminophen	Aspirin
Folic acid	Pyrazinamide	Ampicillin Sodium salt
Abacavir	Cholecalciferol	Nevirapine
Magnesium sulphate	Ritonavir	Ibuprofen
Daruvir	Rifampicin	Ascorbic Acid
Naproxen IP	Metformin	Hydrochlorothiazide
Pantoprazole	Isoniazid	Ferrous Ascorbate
Ergocalciferol	Iron Chloride	Penicillin G Benzathine
Cyclobenzaprine Hydrochloride		

Limitation

1) The test procedure, precautions and interpretation of results for this test must be followed when testing.
2) The following anticoagulants have been validated for use with this test: heparin, EDTA & sodium citrate.
3) Interfering specimens like hemolytic (hemoglobin containing) samples, icteric (bilirubin containing) samples and lipemic samples do not affect the test results.
4) Do not mix reagent from different lots.
5) Interpret faint line as a reactive line. Repeat the test in case of very faint test line or if have any doubt for test line.
6) Although the test is very accurate in detecting HRP2 and /or pLDH, a low incidence of false results can occur. 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.
7) False negative results may arise due to very low parasite density (for instance <100 p/µl), very high parasite density (prozone/hook effect), mutations in the HRP2 gene with deletion of HRP2 antigen, damage by heat, freezing or humidity, application of insufficient volume of blood on the device and use of wrong buffer.
8) False positive results can occur due to various conditions such as rheumatoid factors, antinuclear antibodies, chronic viral infection (hepatitis B or C), parasitic infection (schistosomiasis and trypanosomiasis) and use of wrong buffer.

References

1) Clinical and Laboratory Standards Institute. Procedures and devices for the collection of diagnostic capillary blood specimens; approved standard, fifth edition. CLSI H04-A6, Vol. 28, No. 25, 2008.
2) http://vassarstats.net/clin1.html#def , Richard Lowry.
3) Clinical and Laboratory Standards Institute. Procedures for the collection of diagnostic blood specimens by venipuncture; approved standard, sixth edition. CLSI H03-A6, Vol. 27, No. 26, 2007
4) World Health Organization: Laboratory biosafety manual, third edition. Geneva: WHO; 2004. http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf
5) WHO (2024). World malaria report, World Health Organization, Geneva, Switzerland. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2024
6) Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 8 (2016-2018).
7) Gillet P, Scheirlinck A, Stokx J, De Weggeleire A, Chauque H, Canhanga O, Tadeu B, Mosse C, Tiago A, Mabunda S, Bruggeman C, Bottieau E, Jacobs J: Prozone in malaria rapid diagnostics tests: how many cases are missed? Malar J 2011, 10:166. http://www.malariajournal.com/content/10/1/166
8) Gillet P, Mori M, Van Den Ende J, Jacobs J: Buffer substitution in malaria rapid diagnostic tests causes false positive results. Malar J 2010, 9:215 http://www.malariajournal.com/content/9/1/215
9) Maltha J, Gillet P, Cnops L, Van Den Ende J, Van Esbroeck M, Jacobs J: Malaria rapid diagnostic tests: Plasmodium falciparum infections with high parasite densities may generate false positive Plasmodium vivax pLDH lines. Malar J 2010, 9:198. http://www.malariajournal.com/content/9/1/198
10) Gamboa D, Ho M, Bendezu J, Torres K, Chiodini P, Barnwell J, Incardona S, Perkins M, Bell D, McCarthy J, Cheng Q: 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 2010, 5:e8091. http://www.plosone.org/article/i fo%3Adoi%2F10.1371%2Fjournal.pone.0008091

SYMBOL LEGENDS

Symbol	Explanation of symbol	Symbol	Explanation of symbol
	Consult instructions for use		Contains sufficient for < n > tests
	Non Sterile	REF	Product Code
IVD	In vitro diagnostic medical device	LOT	Lot Number
1°C 40°C	Store at 1-40 °C		Manufacturer
	Caution		Date of manufacture (YYYY-MM)
	Keep dry		Expiration Date (YYYY-MM)
	Do not reuse		Do not use if package is damaged
	Keep away from sunlight		

Product Disclaimer and Warnings

Every warnings and precaution should be taken in to consideration before using the test. Failure to consider “Precaution, Warning and Limitations” may not ensure the diagnostic ability and accuracy of this product. The test result may accordingly be affected by environmental factors and / or user error outside of the control of the Manufacturer and Distributor.

A definitive clinical diagnosis should not be based on the results of a single test, but it should be made by physician after all clinical and laboratory findings have been evaluated.

“In no event shall our company or its distributor be liable for any direct, indirect, punitive, unforeseen, incidental, special consequential damages, to property or life, whatsoever arising out of or connected with an incorrect diagnosis, whether positive or negative, in the use or misuse of this product”.

Manufactured by

Premier Medical Corporation Private Limited

A1-302, GIDC, Sarigam 396155. Dist. Valsad, Gujarat, INDIA.
Customer support E-mail : info@premiermedcorp.com
Tel.: +91 2602780112/113 •Website : www.premiermedcorp.com

• ISO 13485 & EN ISO 13485 Certified Company

Part No.(S)PI16-INS-001, Rev.: AD, Date:2025-09-21 ENGLISH

Note : Instructions for use will be printed in local language of the country using the test, if required.

FIRST RESPONSE® MALARIA Ag. pLDH/HRP2 COMBO CARD TEST

A rapid test for the detection of Malaria pLDH and HRP2 in human whole blood.

1°C 40°C IVD

REF PI16FRC05, PI16FRC10, PI16FRC25, PI16FRC30

Intended Use

First Response® Malaria Ag. pLDH/HRP2 Combo Card Test is intended to be performed by trained users (In either laboratory or point of care settings) as qualitative screening test for detection of *P. falciparum, P. vivax, P. ovale and P. malariae*. The test is intended for use with whole blood specimens (capillary or venous blood). Venous blood with the anti-coagulants heparin, EDTA or citrate do not affect the test results. This kit is not intended to be used for screening of blood donors. The test is not automated and does not require any additional instrument.

Introduction

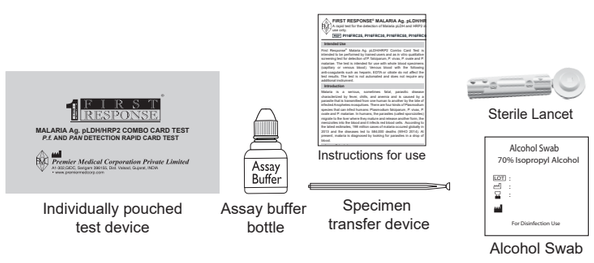
Malaria is a serious, sometimes fatal, parasitic disease characterized by fever, chills, and anemia and is caused by four species of plasmodium parasites that is transmitted from one human to another by the bite of infected Anopheles mosquitoes. There are four Plasmodium species that can infect humans: *Plasmodium falciparum, P. vivax, P. ovale and P. malariae*. In humans, the parasites (called sporozoites) migrate to the liver where they mature and release another form, the merozoites into the blood which infect red blood cells. According to the latest estimates, 263 million cases of malaria occurred globally, and the diseases led to 0.6 million deaths (WHO 2024). At present, malaria is diagnosed by looking for parasites in a drop of blood.

Assay Principle

First Response® Malaria Ag. pLDH/HRP2 Combo Card Test is based on principle of immunochromatography in which nitrocellulose membrane is pre-coated with two monoclonal antibodies as two separate lines. One monoclonal antibody (test line PAN) is PAN specific to lactate dehydrogenase (pLDH) of the *Plasmodium species (Plasmodium falciparum, P. vivax, P. ovale and P. malariae.)* and the other line (test line P.f.) consists of a monoclonal antibody specific to Histidine Rich Protein 2 (HRP2) of the *Plasmodium falciparum.* When the test sample along with assay Buffer flows through the nitrocellulose membrane, monoclonal antibodies conjugated with colloidal gold, which are PAN specific to pLDH and *P. falciparum* specific to HRP2 binds to Plasmodium antigens released from the lysed blood sample. These antigen-conjugated antibody complex moves through the nitrocellulose membrane and binds to corresponding immobilised antibody at test lines, which leads to the formation of colour line / lines indicating reactive results. The control line will appear irrespective of reactive or non reactive sample.

So, the First Response® Malaria Ag. pLDH/HRP2 Combo Card Test is “of additional value” in the differential diagnosis of *Plasmodium falciparum* and other Plasmodium species.

Materials Provided



Individually pouched test device | Assay buffer bottle | Instructions for use | Specimen transfer device | Sterile Lancet | Alcohol Swab

Materials Provided	PI16FRC05	PI16FRC10	PI16FRC25	PI16FRC30
Test Device Pouch Containing: 1 test Device, 1 desiccant	05 Nos.	10 Nos.	25 Nos.	30 Nos.
Specimen transfer device	05 Nos.	10 Nos.	25 Nos.	30 Nos.
Assay buffer bottle	1 No.	1 No.	1 No.	1 No.
Sterile lancet	05 Nos.	10 Nos.	25 Nos.	30 Nos.
Alcohol swab	05 Nos.	10 Nos.	25 Nos.	30 Nos.
Instructions for use	1 No.	1 No.	1 No.	1 No.

Materials Required but Not Provided

- New pair of disposable gloves.
- Permanent marker pen and timer .
- Extra lancets and alcohol swabs, if needed.
- Sharp disposable box and biohazardous waste container.
- Venipuncture blood collection kit (if whole blood is collected by venipuncture).

Storage and Stability

1) First Response® Malaria Ag. pLDH/HRP2 Combo Card Test should be stored at 1 - 40°C.
2) Do not freeze the kit or components.
3) Assay buffer (opened & unopened) & the unopened test device are stable until the expiry date printed on the label, when stored at 1 - 40°C.
4) Test device is sensitive to humidity and heat if remained opened for longer period hence perform the test immediately after removing the test device from the foil pouch.
5) The shelf life of the kit is as indicated on the outer package.

Precautions

1) Wear protective gloves while handling specimens.
2) Dispose of used gloves as biohazardous waste. Wash hands thoroughly afterwards.
3) Avoid splashing or aerosol formation.
4) Clean up spills thoroughly using an appropriate disinfectant.
5) Decontaminate and dispose of all used specimens, test devices, alcohol swabs and specimen transfer device as an infectious waste, in a biohazardous waste container. Dispose of used lancets in a sharp box.

Warnings

1) For *in vitro* diagnostic use only.
2) Read the instructions carefully before performing the test, deviation will invalidate the test results.
3) Apply standard biosafety precautions for handling and disposal of potentially infective material.
4) Assay buffer contains sodium azide as preservative which may be toxic if ingested. When disposed of through sink, flush with large quantity of water.
5) Devices and assay buffer of different lot must not be used.
6) Do not use the test device if the pouch is not intact.
7) Do not use the lancet if the seal is broken.
8) Do not use the test device if the dessicant is missing or if found saturated (orange colour has turned green).
9) Do not smoke, eat or drink while handling specimens and performing a test.
10) Do not re-use the test device, alcohol swab, lancet and specimen transfer device as are intended for single use only.
11) Perform the test by using kit assay buffer, any other buffer or fluid will invalidate the test results.
12) Do not allow the tip of assay buffer bottle to touch specimen well, it contaminate assay buffer.
13) Do not use test device and assay buffer beyond the date of expiry.

Instructions for Use- Single test pack size (Master)

14) Do not use any other specimen other than human whole blood. Do not mix and interchange different specimens.

Specimen collection and storage

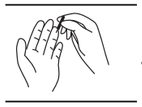
[Collection by venipuncture]

1) Collect the whole blood into the collection tube (containing EDTA/sodium citrate/heparin) by venipuncture.
2) If specimens are not immediately tested (within 1 hour) should be stored at 2-8 °C maximum upto 72 hours (3 days). Using the specimen more than three days can cause non-specific reaction.

Capillary blood specimen collection:

- Wear gloves, massaging the fingertip gently. It will help to obtain a round drop of blood.

- Wipe the complete finger tip with the alcohol swab. Wait untill the finger tip dried completely.



- Detach the protective cap of the lancet and pierce the end of finger tip with the sterile lancet provided.

- Gently squeeze the area until you get enough blood specimen.

- After completion of specimen collection, take the used alcohol swab of same patient and press it on the finger to stop the bleeding.

Note : The lancet should only be used once. Dispose of used lancets in sharp box and used alcohol swab in biohazardous waste container.

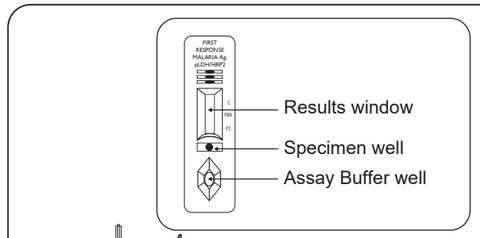
- Do not share used lancets with another person. To prevent possible infection, a used lancet should not be touched by another person.
- Do not use expired lancet. The use of an expired lancet may cause any infection at the punctured skin due to cease to exist its sterility.
- Use new lancet and choose a different puncture site, if repeat the finger prick.
- Do not share used alcohol swab.

Specimen storage

1) Whole blood specimen may be used for testing immediately (within 1 hour) or may be stored at 2-8°C for maximum up to 72 hours (3 days). Do not use blood specimen stored for more than 3 days, It can cause non-specific reaction.

Test Procedure

1) Bring the First Response® Malaria Ag. pLDH/HRP2 Combo Card Test kit components to room temperature (15 - 40°C) prior to 15 minutes of testing.
2) Remove the Test Device and the Specimen transfer Device from the kit and place it on a flat, dry surface and Label the Test Device with specimen identification number/name.
3) Slowly add 5 µl of whole blood to the specimen well using the Specimen Transfer Device. Dispose the used Specimen transfer device as biohazard waste immediately after use.
4) Add two drops of the Assay buffer to the buffer well.
5) Observe for development of colored bands in the Results Window.
6) Interpret test results at 20 minutes. (After recording the results, dispose of test device as a biohazardous waste).
7) Do not interpret after 30 minutes.



① Do not tilt.

Add 5 µl of whole blood to the specimen well.

② Do not tilt.

Add two drops of the Assay buffer to the buffer well.

③ Interpret the test result at 20 minutes after adding assay buffer. Do not read test results after 30 minutes.

Results at 20-30 min.

Caution

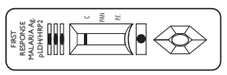
- Hold specimen transfer device and assay buffer vial vertically,else it can lead to inaccurate results.
- Exactly 2 drops of assay buffer should be added. Adding more than or less than 2 drops may cause over flooding or reverse migration phenomenon, which may lead inaccurate results of the test.
- Results can be interpreted any time from 20 to 30 minutes. Do not read test result after 30 minutes. Reading beyond 30 minutes may give inaccurate results. After recording the results, dispose of test device as a biohazard waste.

Internal Quality Control

The visualization of the control line in First Response® Malaria Ag. pLDH/HRP2 Combo Card Test indicates that active ingredient of the strips are functional and the migration is successful. The control line in First Response® Malaria Ag. pLDH/HRP2 Combo Card Test is not meant for specimen addition monitoring.

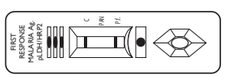
How to Interpret test results

Negative Results



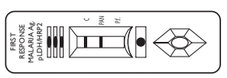
If only one color line appear, at control line 'C' as in the figure, the specimen is negative.

Positive Results



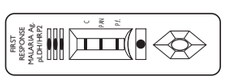
***P.f.* Positive**

If two color lines appears, one at control line 'C' and other at test line *P.f.* as in the figure, the specimen is reactive for antigens to *P.f.* Interpret faint line as reactive line.



PAN Positive

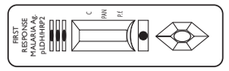
If two color lines appears, one at control line 'C' and other at test line PAN as in the figure, the specimen is reactive for antigens to PAN. Interpret faint line as reactive line.



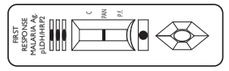
***P.f.* & PAN Positive**

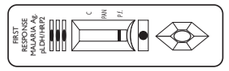
If all three color lines appears, one at control line 'C' and other two at test lines *P.f.* and PAN as in the figure, the specimen is reactive for antigens to *P.f.* and PAN. Interpret faint line as reactive line.

Invalid Results

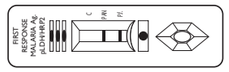


No presence of control line 'C' in the result window (irrespective of presence of test lines) indicates an invalid result.





The directions may not have been followed correctly or the test may have deteriorated.



The Invalid test results should be retested with new test device.

Performance Characteristics

First Response® Malaria Ag.pLDH/HRP2 Combo Card Test were tested using an in-house panel of Positive and Negative clinical specimens characterized by malaria microscopy as the reference method. First Response® Malaria Ag. pLDH/HRP2 Combo Card Test showed 100% sensitivity and 100% specificity. First Response® Malaria Ag. pLDH/HRP2 Combo Card Test showed 100% agreement with the reference method.

Specimen details	Reference Method (Microscopy)		First Response® Malaria Ag. pLDH/HRP2 Combo Card Test		
	Positive	Negative	Positive	Negative	Total
P. falciparum Positive Whole blood specimen	231	0	231	0	231
P. vivax Positive Whole blood specimen	243	0	243	0	243
Malaria Negative Whole blood specimen	0	1287	0	1287	1287
Total	474	1287	474	1287	1761

Reference Method	Specimen details		First Response® Malaria Ag. pLDH/HRP2 Combo Card Test			
	Clinical Status	Parameter	Positive	Negative	Total Result	95% Confidence Interval
Microscopy	*P. falciparum* Positive	Sensitivity	231	00	231	(97.96%-100%)
	P. Vivax Positive	Sensitivity	243	00	243	(98.06%-100%)
	Malaria Negative	Specificity	00	1287	1287	(99.63%-100%)

Note: The product was not been fully assessed with P. ovale & P. malariae and the sensitivity is expected to be low (<50%)

Worldwide Performance Panel

The analytical sensitivity of the First Response® Malaria Ag.pLDH/HRP2 Combo Card Test was carried out by testing WHO worldwide performance panel. Total 10 specimens were tested in-house.The First Response® Malaria Ag.pLDH/HRP2 Combo Card Test showed 100% Sensitivity.

Analytical Sensitivity : In-House Evaluation			
Total Specimens		First Response® Malaria Ag.pLDH/HRP2 Combo Card Test	
		Positive	Negative
05	200 p/µl	05	00
05	2000 p/µl	05	00

Cross Reactivity Study

First Response® Malaria Ag.pLDH/HRP2 Combo Card Test was tested with specimen reactive for other diseases/conditions (mentioned in following table), which may interfere with performance of the test.The First Response® Malaria Ag.pLDH/HRP2 Combo Card Test tested with mentioned specimen for cross reactivity study as well as same diseases/condition specimens# were also used for spiking of malaria positive specimens# to determine effect on sensitivity of the test. None of the specimens interfere with the test results of First Response® Malaria Ag.pLDH/HRP2 Combo Card Test, and showed no cross reactivity with 100% sensitivity.

Specimens tested	*P. falciparum* positive specimens	*P. vivax* positive specimens	Malaria negative specimens	Specimens tested	*P. falciparum* positive specimens	*P. vivax* positive specimens	Malaria negative specimens
Syphilis Positive#	06	06	10	HSV 1/2 Positive#	05	05	05
HIV Positive#	03	03	05	HTLV- I Ab Positive#	07	07	07
Dengue NS1 Positive#	05	05	05	HTLV- II Ab Positive#	09	09	09
Multipara (Pregnant Woman)	08	04	72	HSV - I IgG Positive#	08	08	08
CMV Positive#	03	03	03	Rubella IgG & IgM Positive#	15	15	15
ANA Positive#	04	04	04	HBsAg Positive#	03	03	05
HAV Positive#	04	04	04	Chikungunya Positive#	05	05	05
EBV Positive#	02	02	02	Anti-malarial drug medication	02	02	Not tested
HCV Positive#	03	03	05	Anti-TB drug medication#	03	03	03
Yellow fever virus# post immunization	03	03	04	Infuenza A and B#	03	03	06
Measles#	03	03	04	Visceral leishmaniasis#	03	03	05
Leptospirosis#	03	03	05	Bilirubin#	03	03	05
Cholesterol # Triglycerides	03	03	05	Chagas	03	03	05
Influenza vaccine# recipient	03	03	05	Sickle cell	03	03	05
Leishmaniasis#	03	03	05	Acute hepatitis A infection#	03	03	05
Schistosomiasis#	03	03	05	Toxoplasmosis#	03	03	05

Potential interference substances

The interfering substances that may affect performance of the First Response® Malaria Ag.pLDH/HRP2 Combo Card Test are mentioned in following table. The First Response® Malaria Ag.pLDH/HRP2 Combo Card Test showed no reactivity with any of mentioned specimens and showed 100% specificity. The same specimens# were spiked in malaria positive specimens respectively and tested.First Response® Malaria Ag.pLDH/HRP2 Combo Card Test showed 100% sensitivity with spiked specimens.

Specimens tested	*P. falciparum* positive specimens	*P. vivax* positive specimens	Malaria negative specimens	Specimens tested	*P. falciparum* positive specimens	*P. vivax* positive specimens	Malaria negative specimens
Lipemic specimen#	05	05	05	Low Hematocrit specimens	Not tested	Not tested	05
Icteric specimens#	05	05	05	Whole blood specimen in ACD anticoagulant	03	03	182
Hemolytic specimens	01	01	05	RF Ab Positive#	04	04	09
High Hematocrit specimens	Not tested	Not tested	05	dsDNA Antibody Positive#	01	01	01
Recipient of multiple blood transfusion	03	03	05				

Precision

a) Within run, precision was determined by using 225 replicates of 9 different specimens containing different malaria parasitic count. Within run, precision was observed 100%.
b) Between run, precision was determined by using the 9 different specimens containing different malaria parasitic count in 5 different replicates with 3 different lots of test devices X 5 different days X 3 different sites tested.
Between run, precision was observed 100%.

External Evaluation Report

Place of Evaluation	Year	Sensitivity				Specificity	
		PAN		*P.f.*		PAN	*P.f.*
		200 p/µl	2000 p/µl	200 p/µl	2000 p/µl		
WHO Evaluation Round 6	2014 - 2015	91.4%	100%	82%	100%	100%	100%
Ministry of Health & Children Care Zimbabwe	2014	92.5%		95.5%		98.78%	98.38%
National Public Health & Reference Lab, Ghana	2015	96%		100%		100%	100%

Potential interference drug substances

The details of interference drug molecules are mentioned in the following table. Each interfering drug molecule substance was spiked at the final concentration of 250µg/ml in malaria positive specimens as well as negative specimens, respectively. No false positive or false negative results were observed with any drug molecules, when tested with First Response® Malaria Ag. pLDH/HRP2 Combo Card Test.

Diclofenac	Acetaminophen	Aspirin
Folic acid	Pyrazinamide	Ampicillin Sodium salt
Abacavir	Cholecalciferol	Nevirapine
Magnesium sulphate	Ritonavir	Ibuprofen
Daruvir	Rifampicin	Ascorbic Acid
Naproxen IP	Metformin	Hydrochlorothiazide
Pantoprazole	Isoniazid	Ferrous Ascorbate
Ergocalciferol	Iron Chloride	Penicillin G Benzathine
Cyclobenzaprine Hydrochloride		

Limitation

1) The test procedure, precautions and interpretation of results for this test must be followed when testing.
2) The following anticoagulants have been validated for use with this test: heparin, EDTA & sodium citrate.
3) Interfering specimens like hemolytic (hemoglobin containing) samples, icteric (bilirubin containing) samples and lipaemic samples do not affect the test results.
4) Do not mix reagent from different lots.
5) Interpret faint line as a reactive line. Repeat the test in case of very faint test line or if have any doubt for test line.
6) Although the test is very accurate in detecting HRP2 and /or pLDH, a low incidence of false results can occur. 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.
7) False negative results may arise due to very low parasite density (for instance <100 p/µl), very high parasite density (prozone/hook effect), mutations in the HRP2 gene with deletion of HRP2 antigen, damage by heat, freezing or humidity, application of insufficient volume of blood on the device and use of wrong buffer.
8) False positive results can occur due to various conditions such as rheumatoid factors, antinuclear antibodies, chronic viral infection (hepatitis B or C), parasitic infection (schistosomiasis and trypanosomiasis) and use of wrong buffer.

References

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SYMBOL LEGENDS

Symbol	Explanation of symbol	Symbol	Explanation of symbol
	Consult instructions for use	Σ	Contains sufficient for < n > tests
	Non Sterile	REF	Product Code
IVD	In vitro diagnostic medical device	LOT	Lot Number
1°C 40°C	Store at 1-40 °C		Manufacturer
	Caution		Date of manufacture (YYYY-MM)
	Keep dry		Expiration Date (YYYY-MM)
	Do not reuse		Do not use if package is damaged
	Keep away from sunlight		

Product Disclaimer and Warnings

Every warning and precaution should be taken in to consideration before using the test. Failure to consider “Precaution, warning and Limitations” may not ensure the diagnostic ability and accuracy of this product. The test result may accordingly be affected by environmental factor and / or user error outside of the control of the Manufacturer and Distributor.

A definitive clinical diagnosis should not be based on the results of a single test, but it should be made by physician after all clinical and laboratory findings have been evaluated.

“In no event shall our comapny or its distributor be liable for any direct, indirect, punitive, unforeseen, incidental, special consequential damages, to property or life, whatsoever arising out of or connected with an incorrect diagnosis, whether positive or negative, in the use or misuse of this product”.

Manufactured by

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Customer support E-mail : info@premiermedcorp.com
Tel.: +91 2602780112/113 •Website : www.premiermedcorp.com

• ISO 13485 & EN ISO 13485 Certified Company

Part No.(S)PI16-INS-003, Rev.: AD, Date:2025-09-21 ENGLISH

Note : Instructions for use will be printed in local language of the country using the test, if required.

FIRST RESPONSE® MALARIA Ag. pLDH/HRP2 COMBO CARD TEST

A rapid test for the detection of Malaria pLDH and HRP2 in human whole blood.

1°C 40°C IVD

REF PI16FRC10s & PI16FRC25s

Intended Use

First Response® Malaria Ag. pLDH/HRP2 Combo Card Test is intended to be performed by trained users (In either laboratory or point of care settings) as qualitative screening test for detection of P. falciparum, P. vivax, P. ovale and P. malariae. The test is intended for use with whole blood specimens (capillary or venous blood). Venous blood with the anti-coagulants heparin, EDTA or citrate do not affect the test results. This kit is not intended to be used for screening of blood donors. The test is not automated and does not require any additional instrument.

Introduction

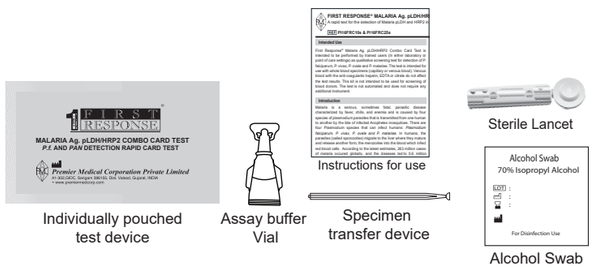
Malaria is a serious, sometimes fatal, parasitic disease characterized by fever, chills, and anemia and is caused by four species of plasmodium parasites that is transmitted from one human to another by the bite of infected Anopheles mosquitoes. There are four Plasmodium species that can infect humans: *Plasmodium falciparum, P. vivax, P. ovale and P. malariae*. In humans, the parasites (called sporozoites) migrate to the liver where they mature and release another form, the merozoites into the blood which infect red blood cells. According to the latest estimates, 263 million cases of malaria occured globally, and the diseases led to 0.6 million deaths (WHO 2024). At present, malaria is diagnosed by looking for parasites in a drop of blood.

Assay Principle

First Response® Malaria Ag. pLDH/HRP2 Combo Card Test is based on principle of immunochromatography in which nitrocellulose membrane is pre-coated with two monoclonal antibodies as two separate lines. One monoclonal antibody (test line PAN) is PAN specific to Lactate dehydrogenase (pLDH) of the *Plasmodium species (Plasmodium falciparum, P. vivax, P. ovale and P. malariae.)* and the other line (test line P.f.) consists of a monoclonal antibody specific to Histidine Rich Protein 2 (HRP2) of the *Plasmodium falciparum.* When the test sample along with assay Buffer flows through the nitrocellulose membrane, monoclonal antibodies conjugated with colloidal gold, which are PAN specific to pLDH and *P. falciparum* specific to HRP2 binds to Plasmodium antigens released from the lysed blood sample. These antigen-conjugated antibody complex moves through the nitrocellulose membrane and binds to corresponding immobilised antibody at test lines, which leads to the formation of colour line / lines indicating reactive results. The control line will appear irrespective of reactive or non reactive sample.

So, the First Response® Malaria Ag. pLDH/HRP2 Combo Card Test is “of additional value” in the differential diagnosis of *Plasmodium falciparum* and other Plasmodium species.

Materials Provided



Materials Provided	PI16FRC10s	PI16FRC25s
Each single test pack contents: (Test device with desiccant, specimen transfer device) alcohol swab,sterile lancet, buffer vial & condensed instructions for use)	10 Nos.	25 Nos.
Master instructions for use	1 No.	1 No.

Materials Required but Not Provided

- New pair of disposable gloves.
- Permanent marker pen and timer .
- Extra lancets and alcohol swabs, if needed.
- Sharp disposable box and biohazardous waste container.
- Venipuncture blood collection kit (if whole blood is collected by venipuncture).

Storage and Stability

1) First Response® Malaria Ag. pLDH/HRP2 Combo Card Test should be stored at 1 - 40°C.
2) Do not freeze the kit or components.
3) Unopened test device & single use buffer vial are stable until the expiry date printed on the label, when stored at 1 - 40°C.
4) The device is sensitive to humidity and heat if remained opened for longer period hence perform the test immediately after removing the test device from the foil pouch.
5) The shelf life of the kit is as indicated on the outer package.

Precautions

1) Wear protective gloves while handling specimens.
2) Dispose of used gloves as biohazardous waste. Wash hands thoroughly afterwards.
3) Avoid splashing or aerosol formation.
4) Clean up spills thoroughly using an appropriate disinfectant.
5) Decontaminate and dispose of all used specimens, test devices, alcohol swabs and specimen transfer device as an infectious waste, in a biohazardous waste container. Dispose of used lancets in a sharp box.

Warnings

1) For in vitro diagnostic use only.
2) Read the instructions carefully before performing the test, deviation will invalidate the test results.
3) Apply standard biosafety precautions for handling and disposal of potentially infective material.
4) Assay buffer contains sodium azide as preservative which may be toxic if ingested. When disposed of through sink, flush with large quantity of water.
5) Devices and assay buffer of different lot must not be used.
6) Do not use the test device if the pouch is not intact.
7) Do not use the lancet if the seal is broken.
8) Do not use the test device if the desiccant is missing or if found saturated (orange colour has turned green).
9) Do not smoke, eat or drink while handling specimens and performing a test.
10) Do not re-use the test device, alcohol swab, lancet and specimen transfer device as are intended for single use only.
11) Perform the test by using kit assay buffer, any other buffer or fluid will invalidate the test results.
12) Do not use test device and assay buffer beyond the date of expiry.
13) Do not eat the desiccant.

Instructions for Use- Single test pack size (Condensed)

First Response® Malaria Ag. pLDH / HRP2 Combo Card Test

Rapid One Step Malaria Ag.pLDH and HRP2 detection in human whole blood

1°C - 40°C | IVD

REF PI16FRC10s & PI16FRC25s

Intended Use :

First Response® Malaria Ag. pLDH/HRP2 Combo Card Test is intended to be performed by trained users (In either laboratory or point of care settings) as qualitative screening test for detection of *P. falciparum, P. vivax, P. ovale and P. malariae.* The test is intended to be use with whole blood specimens (capillary or venous blood). Venous blood with the anti-coagulants heparin, EDTA or Citrate do not affect the test results. This kit is not intended to be used for screening of blood donors. The test is not automated and does not required any additional instrument.

Materials provided :

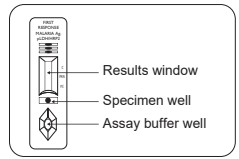
Test device with desiccant, specimen transfer device, assay buffer vial,sterile lancet,alcohol swab,instruction for use

Storage & stability :

1) First Response® Malaria Ag. pLDH/HRP2 Combo Card Test should be stored at 1°C - 40°C.
2) Test device is sensitive to humidity and heat if remained opened for longer period hence perform the test immediately after removing the test device from the foil pouch.
3) The shelf life of the kit is as indicated on the outer package.

Precautions & warnings:

1) Wear protective gloves while handling specimens.
2) Apply standard biosafety precautions for handling and disposal of potentially infective material.
3) Assay buffer contains sodium azide as preservative which may be toxic if ingested. When disposed of through sink, flush with large quantity of water.
4) Do not use the test device if the pouch is not intact.
5) Do not use the lancet if the seal is broken.
6) Do not use the test device if the desiccant is missing or if found saturated (orange colour has turned green).
7) Do not re-use the test device, alcohol swab, lancet and specimen transfer device as are intended for single use only.
8) Perform the test by using kit assay buffer, any other buffer or fluid will invalidate the test results.
9) Do not eat the desiccant.
10) Do not use any other specimen other than human whole blood & do not mix and interchange different specimens.



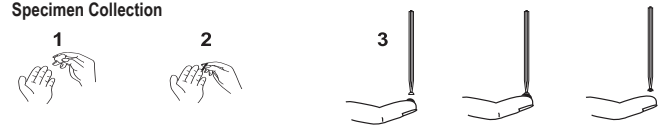
Test Procedure :

1) Bring the First Response® Malaria Ag. pLDH/HRP2 Combo Card Test kit components to room temperature (15 - 40°C) prior to 15 minutes of testing.
2) Remove the test Device and the specimen transfer device from the kit and place it on a flat, dry surface and label the test device with specimen identification number/name.
3) Slowly add 5 µl of whole blood to the specimen well using the specimen transfer device. Dispose the used Specimen transfer device as biohazardous waste immediately after use.
4) Add two drops of the assay buffer to the assay buffer well.
5) Observe for development of colored bands in the Results Window.
6) Interpret test results at 20 minutes. (After recording the results, dispose of test device as a biohazardous waste).
7) Do not interpret after 30 minutes.

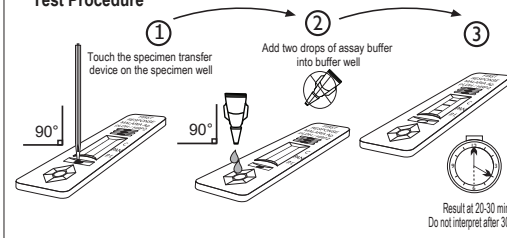
Assay buffer vial opening process

Specimen Collection , Test Procedure & Result Interpretation

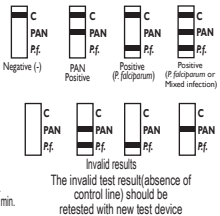
Specimen Collection



Test Procedure



Results Interpretation



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