WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: First Response® Malaria Antigen P. *falciparum* (HRP2) Card Test
WHO reference number: PQDx 0283-010-00

First Response® Malaria Antigen P. *falciparum* (HRP2) Card Test with product codes **PI13FRC25s, PI13FRC10s, PI13FRC25,** and **PI13FRC30**, manufactured by **Premier Medical Corporation 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 First Response® Malaria Ag. pLDH/HRP2 Combo Card Test

	Date	Outcome
Prequalification listing	04 December 2018	listed
Dossier assessment	28 September 2018	MR
Site inspection(s) of quality management system	08 October 2018	MR
Product performance evaluation	2015	MR

MR: Meets Requirements

Intended use

According to the claim from manufacturer for product '*First Response® Malaria Antigen P. falciparum (HRP2) Card Test is intended to be performed by trained users (in either laboratory and point of care settings) as qualitative screening in vitro diagnostic test for detection of P. falciparum specific HRP2 antigens. The test is intended for use with human whole blood specimens (capillary or venous blood). Venous blood with the anti-coagulants heparin, EDTA, or sodium citrate does 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.*'

Assay description

According to the claim from manufacturer for product '*First Response® Malaria Antigen P. falciparum (HRP2) Card Test is based on principle of immunochromatography in which nitrocellulose membrane is pre-coated with one monoclonal antibody (test line P.f.) specific to Histidine Rich Protein 2 (HRP2) of the Plasmodium falciparum. When the test sample along with buffer flow through the nitrocellulose membrane, second monoclonal antibodies specific for HRP2 Antigen conjugated with colloidal gold, binds to HRP2 antigens released from the lysed blood sample. This antigen-conjugated antibody complex moves through the nitrocellulose membrane and binds to the immobilized HRP2 specific*

monoclonal antibody at the test line, which leads to the formation of colour band indicating reactive results. The control band will appear irrespective of reactive or non-reactive sample. So, the First Response® Malaria Antigen P. falciparum (HRP2) Card Test is designed for the diagnosis of Plasmodium falciparum.'

Test kit contents

Configuration	Product code	Description
25 × multi kit	PI13FRC25	**Materials provided:** 1. Individually pouched test device 2. Assay buffer bottle 3. Instructions for Use 4. Sterile lancets 5. Specimen transfer device 6. Alcohol swabs **Items required but not provided:** 1. New pair of disposable gloves 2. Permanent marker pen and timer 3. Extra lancets and alcohol swabs, if needed 4. Sharp disposable box and biohazardous waste container 5. Venipuncture blood collection kit (if whole blood is collected by venipuncture)
30 × multi kit	PI13FRC30	
25 × single kit	PI13FRC25s	**Materials provided:** 1. Test device with desiccant 2. Specimen transfer device 3. Assay buffer vial 4. Sterile lancet 5. Alcohol swab 6. Instruction for use
10 × single kit	PI13FRC10s	

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 6, First Response® Malaria Antigen P. falciparum (HRP2) Card Test was given priority for WHO prequalification.

Dossier assessment

Premier Medical Corporation Limited submitted a product dossier for First Response® Malaria Antigen P. *falciparum* (HRP2) 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 28 September 2018.

Commitments for prequalification

The manufacturer was requested in the stage following the Dossier Review to provide an amended IFU that:

- Reflects the intended use of the product (i.e. that testing is not intended to include blood donors).
- Clearly reports investigation of potentially cross-reacting conditions and interfering substances.

The manufacturer is requested to demonstrate implementation of the revised IFU by March 2019.

Based on the product dossier screening and assessment findings, the product dossier for First Response® Malaria Antigen P. *falciparum* (HRP2) Card Test meets WHO prequalification requirements.

Manufacturing site inspection

A comprehensive inspection was performed at the sites of manufacture Premier Medical Corporation Limited (site 1: A1-302, GIDC, Sarigam 396 155, Valsad, Gujarat, India and site 2: 32-35A, Shree Ganesh Industrial Estate, Kachigam, Nani Daman, Daman 396215, India) of First Response® Malaria Antigen P. *falciparum* (HRP2) Card Test between 12-14 March 2018 as per the "*Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics*" (PQDx_014). The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality.

The manufacturer's responses to the nonconformities found at the time of the inspection were accepted on 8 October 2018.

Based on the site inspection and corrective action plan review, the quality management system for First Response® Malaria Antigen P. *falciparum* (HRP2) 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 (91.0% at 200 parasites/µl), false-positive rates (1.0% for clean negatives, 0.0% for P. *vivax* at 200 parasites/µl, 0.0% for P. *vivax* at 2000 to 5000 parasites/µl) and invalid rate (0.0%), First Response® Malaria Antigen P. *falciparum* (HRP2) 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**	
	P*f*	**P*v***	**P*f***	**P*v***		
First Response® Malaria Antigen P. *falciparum* (HRP2) Card Test	91.0	NA	NA	0.0	1.0	0.0

Labelling

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

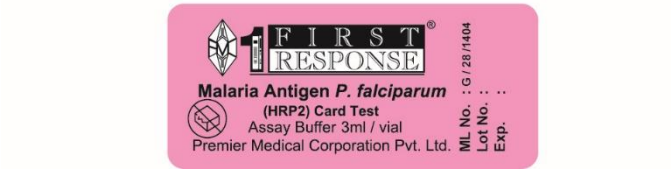
1. Labels

Bulk pack-25 test/kit

Assay buffer label- Malaria HRP2 card Test

Bulk pack-30 test/kit

Assay buffer label- Malaria HRP2 card Test

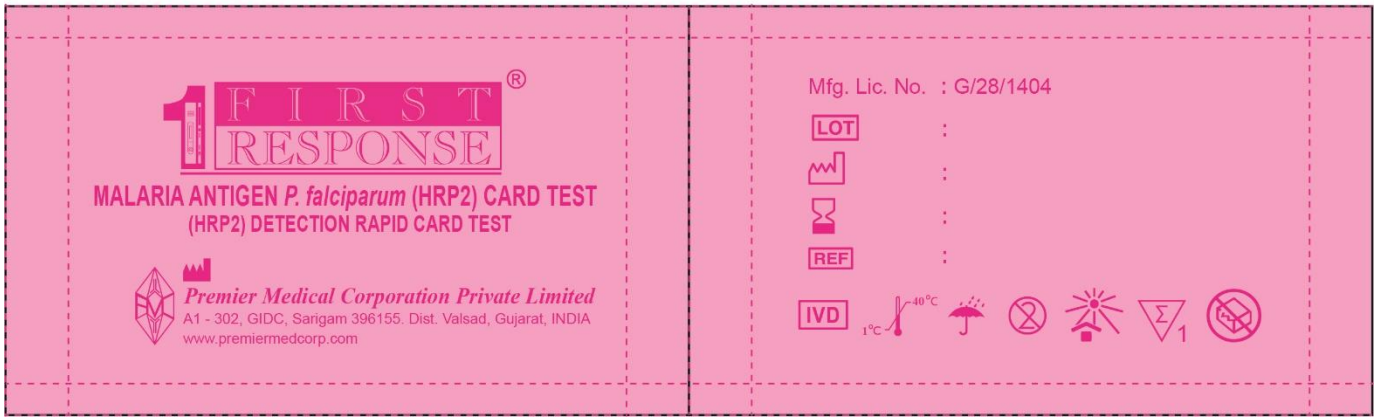


Single pack- (10 test/kit; 25 test/kit)

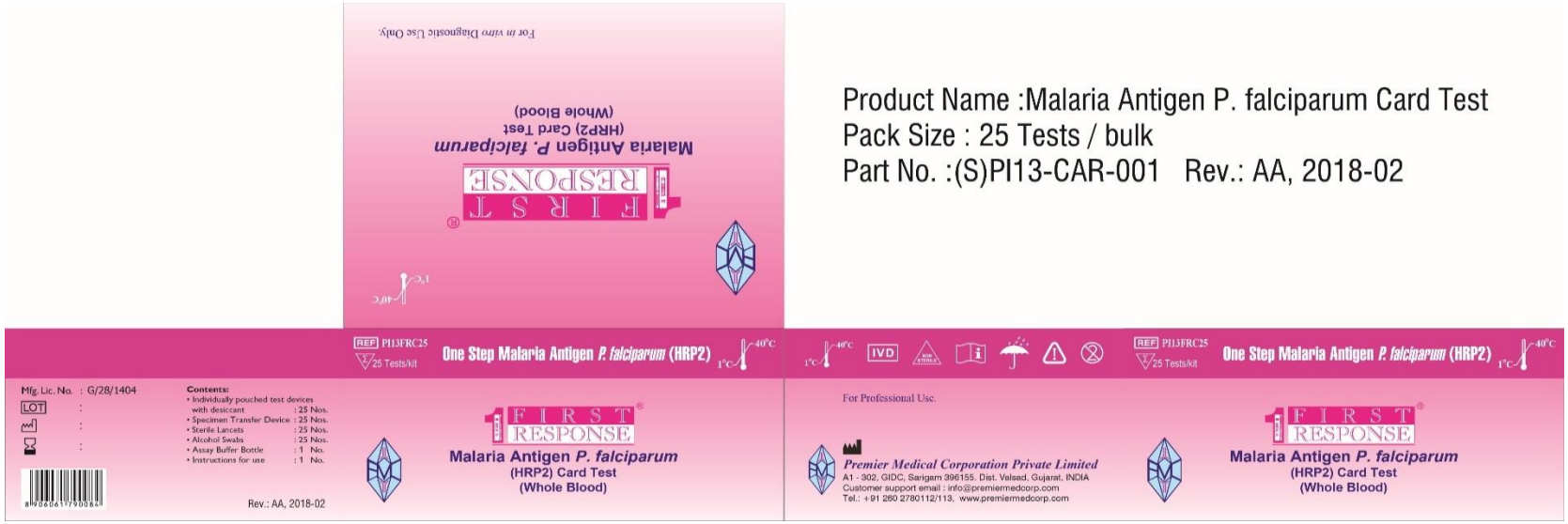
Assay buffer vial for Malaria (HRP2) Card Test

Pouch for all Pack sizes:

Pouch for F.R Malaria HRP2 Card Test



Carton for Bulk Pack- 25 test/kit



Carton for Bulk Pack- 30 test/kit

Carton for single Pack- 10 test/kit

Carton for single Pack- 25 test/kit

2. Instructions for use

Bulk pack size

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, 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.

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
	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

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) 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.
3) World Health Organization: Laboratory biosafety manual, third edition. Geneva: WHO; 2004. http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf
4) WHO (2014). World malaria report, World Health Organization, Geneva, Switzerland. http://rbm.who.int/wmr.2014.
5) Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 5 (2013).
6) Gillet P, Scheirlinck A, Stokx J, De Weggeleire A, Chauque H, Canhanga O, Tadeu B, Mosse C, Tiago A, Mabunda S, Bruggeman C, Bottleau 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
7) 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
8) 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
9) 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

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
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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)PI13-INS-001, Rev.: AC ENGLISH

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

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FIRST RESPONSE® MALARIA ANTIGEN *P. falciparum* (HRP2) CARD TEST

A rapid test for the detection of *P. falciparum* specific HRP2 antigen in human whole blood.

1°C - 40°C IVD

REF **PI13FRC25, PI13FRC30, PI13FRC50, PI13FRC60 & PI13FRC100**

Intended Use

First Response® Malaria Antigen P. falciparum (HRP2) Card Test is intended to be performed by trained users (In either laboratory or point of care settings) as qualitative screening in vitro diagnostic test for detection of *P. falciparum* specific HRP2 antigens. The test is intended for use with human 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.

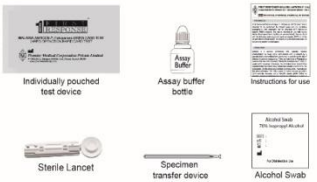
Introduction

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, 198 million cases of malaria occured globally in 2013 and the diseases led to 584,000 deaths (WHO 2014). At present, malaria is diagnosed by looking for parasites in a drop of blood.

Assay Principle

First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test is based on principle of immunochromatography in which nitrocellulose membrane is pre-coated with one monoclonal antibody (test line P.f.) specific to Histidine Rich Protein 2 (HRP2) of the *Plasmodium falciparum*. When the test sample along with buffer flows through the nitrocellulose membrane, second monoclonal antibodies specific for HRP2 Antigen conjugated with colloidal gold, binds to HRP2 antigens released from the lysed blood sample. This antigen-conjugated antibody complex moves through the nitrocellulose membrane and binds to the immobilised HRP2 specific monoclonal antibody at the test line, which leads to the formation of colour band indicating reactive results. The control band will appear irrespective of reactive or non reactive sample. So, the First Response®Malaria Antigen *P. falciparum* (HRP2) Card Test is designed for the diagnosis of *Plasmodium falciparum*.

Materials Provided



Materials Provided	PI13FRC25	PI13FRC30	PI13FRC50	PI13FRC60	PI13FRC100
Test Device Pouch Containing 1 test Device, 1 desiccant	25 Nos.	30 Nos.	50 Nos.	60 Nos.	100 Nos.
Specimen transfer device	25 Nos.	30 Nos.	50 Nos.	60 Nos.	100 Nos.
Assay buffer bottle	1 No.	1 No.	2 Nos.	4 Nos.	4 Nos.
Sterile lancet	25 Nos.	30 Nos.	50 Nos.	60 Nos.	100 Nos.
Alcohol swab	25 Nos.	30 Nos.	50 Nos.	60 Nos.	100 Nos.
Instructions for use	1 No.	1 No.	1 No.	1 No.	2 Nos.

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 Antigen P. falciparum (HRP2) Card Test should be stored at 1°C - 40°C.
2) Do not freeze the kit or components.
3) Unopened test device & buffer bottle 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 biohazard 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 found saturated.
9) Do not smoke, eat or drink while handling specimens and performing a test.

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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 will contaminate assay buffer.
13) Do not use test device and assay buffer beyond the date of expiry.
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 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 : A lancet should only be used once. Dispose of used lancets in sharp box and alcohol swab in biohazard waste container.

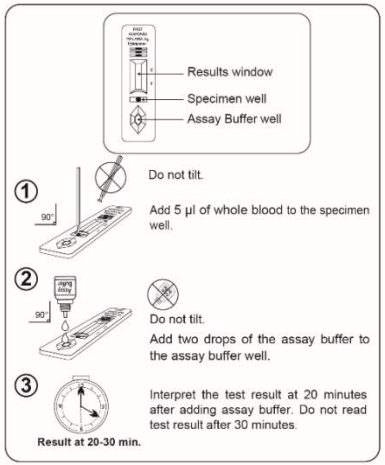
- Do not share used lancets with another person. To prevent possible infection, a used lancet should not be touched by another person.
- Do not use expired lancet. The use of an expired lancet may cause any infection at the punctured skin due to cease to exist its sterility.
- Use new lancet and choose 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-sepcific reaction.

Test Procedure

1) Bring the First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test 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 immidiately 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.



Caution

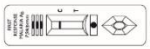
- Hold specimen transfer device and assay buffer bottle verticaly,else it can lead to inaccurate results.
- Exactly 2 drops of assay buffer should be added. Adding more or less than 2 drops may cause over flooding or reverse migration phenomenon, which may lead inaccurate results of the test.
- Results 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 Antigen *P. falciparum* (HRP2) Card Test indicates that active ingredient of the strips are functional and the migration is successful. The control line in First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test is not meant for specimen addition monitoring.

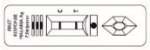
How to Interpret test results

Negative Results



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

Positive Results

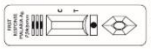


If two color lines appears, one at control line 'C' and other at test line 'T' as in the figure, the specimen is reactive for antigens to *P.f.*

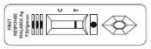
P.f. **Positive**

Interprete faint line as reactive line

Invalid Results



No presence of control line 'C' in the result window (irrespective of presense 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 Antigen *P. falciparum* (HRP2) 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 Antigen *P. falciparum* (HRP2) Card Test showed 100% sensitivity and 100% specificity. First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test showed 100% agreement with the reference method.

Specimen details	Reference Method (Microscopy)		First Response® Malaria Antigen *P. falciparum*(HRP2)Card Test		
	Positive	Negative	Positive	Negative	Total
P. falciparum Positive Whole blood specimen	224	0	224	0	224
Malaria Negative Whole blood specimen	0	963	0	963	963
Total	224	963	224	963	1187

Reference Method	Specimen details		First Response® Malaria Antigen *P. falciparum*(HRP2)Card Test			
	Clinical Status	Parameter	Positive	Negative	Total Result	95% Confidence Interval
Microscopy	*P. falciparum* Positive	Sensitivity	224	00	224	(97.89-%-100%)
	Malaria Negative	Specificity	00	963	963	(99.50%-100%)

Worldwide Performance Panel

The analytical sensitivity of the First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test was carried out by testing WHO worldwide performance panel. Total 10 specimens were tested in-house. The First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test showed 100% sensitivity.

Analytical Sensitivity : In-House Evaluation			
Total Specimens		First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test	
		Positive	Negative
05	200 p/µl	05	00
05	2000 p/µl	05	00

Cross Reactivity Study

First Response® Malaria Antigen *P. falciparum* (HRP2) 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 Antigen *P. falciparum* (HRP2) 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 Antigen *P. falciparum* (HRP2) Card Test, and showed no cross reactivity with 100% sensitivity.

Specimen Details	Specimen Size	Specimen Details	Specimen Size
Syphilis Positive#	05	HSV 1/2 Positive#	05
HIV Positive#	05	Rubella Positive#	05
Dengue NS1 Positive#	05	HTLV- I Ab Positive#	07
Pregnant Woman	03	HTLV- II Ab Positive#	09
CMV Positive#	03	HSV - I IgG Positive#	08
ANA Positive#	04	Rubella IgG Positive#	10
HAV Positive#	04	HBV Positive#	05
EBV Positive#	02	Chikungunya Positive#	05
HCV Positive#	05	Anti-malarial drug medication	03
RF Positive	05	Anti-TB drug medication#	03
P.Vivax Positive*	115		

*Note: Specimens were not used for spiking

Potential interference substances

The interfering substances that may affect performance of the First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test are mentioned in following table. The First Response® Malaria Antigen *P. falciparum* (HRP2) 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 Antigen *P. falciparum* (HRP2) Card Test showed 100% sensitivity with spiked specimens.

Specimen details	Specimen size	Specimen details	Specimen size
Lipemic specimen#	05	Low Hematocrit specimens	05
Icteric specimens#	05	Whole blood specimen in ACD anticoagulant	05
Hemolytic specimens	05	RF Ab 4001 - 5000 IU/mL Plasma†	04
High Hematocrit specimens	05	dsDNA Antibody Positive Plasma‡	01

Precision

a) Within run, precision was determined by using 81 replicates of 15 different specimens containing different malaria parasitic count. Within run, precision was observed 100%.
b) Between run, precision was determined by using the 15 different specimens containing different malaria parasitic count in 3 different replicates with 3 different lots of test devices X 3 different persons X 3 different sites tested. Between run, precision was observed 100%.

External Evaluation Report

Name of the Institute	Year of Testing	Sensitivity		Specificity
		200 P/µl	2000 P/µl	
WHO Evaluation Round 6	2014-2015	91%	100%	99.0%
National Public Health & Reference Lab, Ghana	2015	100%		100%
Ministry of Health and Children Care, Zimbabwe	2014	95.5 %		98%

Single test pack size- (Master)

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, 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.

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

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) 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
3) World Health Organization: Laboratory biosafety manual, third edition. Geneva: WHO; 2004. http://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf
4) WHO (2014). World malaria report, World Health Organization, Geneva, Switzerland. http://rbm.who.int/wmr.2014.
5) Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 5 (2013).
6) 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
7) 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
8) 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
9) 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

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)PI13-INS-003, Rev.: AC ENGLISH
Note : Instructions for use will be printed in local language of the country using the test, if required.

④

FIRST RESPONSE® MALARIA ANTIGEN *P. falciparum* (HRP2) CARD TEST

A rapid test for the detection of *P. falciparum* specific HRP2 antigen in human whole blood.

1°C 40°C IVD

REF **PI13FRC10s & PI13FRC25s**

Intended Use

First Response® Malaria Antigen P. falciparum (HRP2) Card Test is intended to be performed by trained users (In either laboratory or point of care settings) as qualitative screening in vitro diagnostic test for detection of *P. falciparum* specific HRP2 antigens. The test is intended for use with human 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, 198 million cases of malaria occured globally in 2013 and the diseases led to 584,000 deaths (WHO 2014). At present, malaria is diagnosed by looking for parasites in a drop of blood.

Assay Principle

First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test is based on principle of immunochromatography in which nitrocellulose membrane is pre-coated with one monoclonal antibody (test line P.f.) specific to Histidine Rich Protein 2 (HRP2) of the *Plasmodium falciparum*. When the test sample along with buffer flows through the nitrocellulose membrane, second monoclonal antibodies specific for HRP2 antigen conjugated with colloidal gold, binds to HRP2 antigens released from the lysed blood sample. This antigen-conjugated antibody complex moves through the nitrocellulose membrane and binds to the immobilised HRP2 specific monoclonal antibody at the test line, which leads to the formation of colour band indicating reactive results. The control band will appear irrespective of reactive or non reactive sample. So, the First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test is designed for the diagnosis of *Plasmodium falciparum*.

Materials Provided

Materials Provided	PI13FRC10s	PI13FRC25s
Each single test pack contents: [(Test device , 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 Antigen *P. falciparum* (HRP2) Card Test should be stored at 1°C - 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) 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 biohazard 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 found saturated.
9) Do not smoke, eat or drink while handling specimens and performing a test.

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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 vial to touch specimen well, it contaminate assay buffer.
13) Do not use test device and assay buffer beyond the date of expiry.
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 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 : A lancet should only be used once. Dispose of used lancets in sharp box and alcohol swab in biohazard waste container.

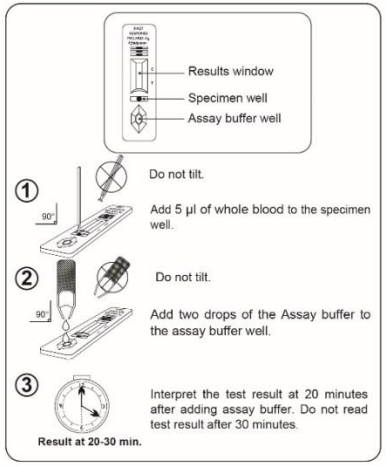
- Do not share used lancets with another person. To prevent possible infection, a used lancet should not be touched by another person.
- Do not use expired lancet. The use of an expired lancet may cause any infection at the punctured skin due to cease to exist its sterility.
- Use new lancet and choose 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-sepcific reaction.

Test Procedure

1) Bring the First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test 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 pouch 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 immidiately 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.



Caution

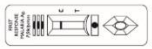
- Hold specimen transfer device and assay buffer vial, verticaly,else it can lead to inaccurate results.
- Exactly 2 drops of assay buffer should be added. Adding more or less than 2 drops may cause over flooding or reverse migration phenomenon, which may lead inaccurate results of the test.
- Results 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 Antigen *P. falciparum* (HRP2) Card Test indicates that active ingredient of the strips are functional and the migration is successful. The control line in First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test is not meant for specimen addition monitoring.

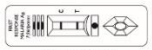
How to Interpret test results

Negative Results



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

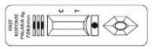
Positive Results



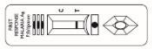
P.f. Positive

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.
Interprete faint line as reactive line

Invalid Results



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



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

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

Performance Characteristics

First Response® Malaria Antigen *P. falciparum* (HRP2) 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 Antigen *P. falciparum* (HRP2) Card Test showed 100% sensitivity and 100% specificity. First Response® Malaria Antigen P. falciparum (HRP2) Card Test showed 100% agreement with the reference method.

Specimen details	Reference Method (Microscopy)		First Response® Malaria Antigen *P. falciparum*(HRP2)Card Test		
	Positive	Negative	Positive	Negative	Total
P. falciparum Positive Whole blood specimen	224	0	224	0	224
Malaria Negative Whole blood specimen	0	963	0	963	963
Total	224	963	224	963	1187

Reference Method	Specimen details		First Response® Malaria Antigen *P. falciparum*(HRP2)Card Test			
	Clinical Status	Parameter	Positive	Negative	Total Result	95% Confidence Interval
Microscopy	*P. falciparum* Positive	Sensitivity	224	00	224	(97.89-%-100%)
	Malaria Negative	Specificity	00	963	963	(99.50%-100%)

Worldwide Performance Panel

The analytical sensitivity of the First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test was carried out by testing WHO worldwide performance panel. Total 10 specimens were tested in-house. The First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test showed 100% sensitivity.

Analytical Sensitivity : In-House Evaluation			
Total Specimens		First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test	
		Positive	Negative
05	200 p/µl	05	00
05	2000 p/µl	05	00

Cross Reactivity Study

First Response® Malaria Antigen *P. falciparum* (HRP2) 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 Antigen *P. falciparum* (HRP2) 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 Antigen *P. falciparum* (HRP2) Card Test, and showed no cross reactivity with 100% sensitivity.

Specimen Details	Specimen Size	Specimen Details	Specimen Size
Syphilis Positive[z]	05	HSV 1/2 Positive[z]	05
HIV Positive[#]	05	Rubella Positive[#]	05
Dengue NS1 Positive[c]	05	HTLV- I Ab Positive[k]	07
Pregnant Woman	03	HTLV- II Ab Positive[a]	09
CMV Positive[a]	03	HSV - I IgG Positive[a]	08
ANA Positive[#]	04	Rubella IgG Positive[#]	10
HAV Positive[a]	04	HBV Positive[#]	05
EBV Positive[c]	02	Chikungunya Positive[z]	05
HCV Positive[a]	05	Anti-malarial drug medication	03
RF Positive	05	Anti-TB drug medication[c]	03
P.Vivax Positive*	115		

*Note: Specimens were not used for spiking

Potential interference substances

The interfering substances that may affect performance of the First Response® Malaria Antigen P. falciparum (HRP2) Card Test are mentioned in following table. The First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test showed no reactivity with any of mentioned specimens and showed 100% specificity. The same specimens[z] were spiked in malaria positive specimens respectively and tested.First Response® Malaria Antigen *P. falciparum* (HRP2) Card Test showed 100% sensitivity with spiked specimens.

Specimen details	Specimen size	Specimen details	Specimen size
Lipemic specimen[a]	05	Low Hematocrit specimens	05
Icteric specimens[a]	05	Whole blood specimen in ACD anticoagulant	05
Hemolytic specimens	05	RF Ab 4001 - 5000 IU/mL Plasma[k]	04
High Hematocrit specimens	05	dsDNA Antibody Positive Plasma[a]	01

Precision

a) Within run, precision was determined by using 81 replicates of 15 different specimens containing different malaria parasitic count. Within run, precision was observed 100%.
b) Between run, precision was determined by using the 15 different specimens containing different malaria parasitic count in 3 different replicates with 3 different lots of test devices X 3 different persons X 3 different sites tested.
Between run, precision was observed 100%.

External Evaluation Report

Name of the Institute	Year of Testing	Sensitivity		Specificity
		200 P/ul	2000 P/ul	
WHO Evaluation Round 6	2014-2015	91%	100%	99.0%
National Public Health & Reference Lab, Ghana	2015	100%		100%
Ministry of Health and Children Care, Zimbabwe	2014	95.5 %		98%

Single test pack size- (Condensed)

First Response Malaria Antigen *P. falciparum* (HRP2) Card Test

Rapid One Step Malaria Antigen *P. falciparum* (HRP2) Test

1°C 40°C IVD

REF PI13FRC10s & PI13FRC25s

Intended Use :

First Response® Malaria Antigen P. falciparum (HRP2) Card Test is intended to be performed by trained users (In either laboratory or point of care settings) as qualitative screening in vitro diagnostic test for detection of P. falciparum specific HRP2 antigens. The test is intended for use with human 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.

Materials provided :

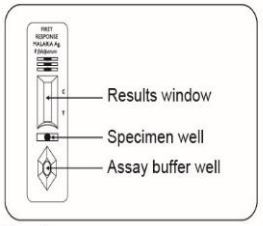
Test device with desiccant & specimen transfer device, assay buffer vial,sterile lancet,alcohol swab,instruction for use

Storage & stability :

1) First Response® Malaria Antigen P. falciparum (HRP2) 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:

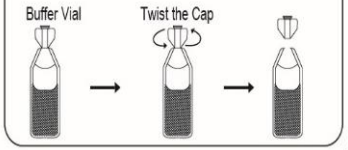
1) Wear protective gloves while handling specimens.
2) Apply standard biosafety precautions for handling and disposal of potentially infective material.
3) Assay buffer contains sodium azide as preservative which may be toxic if ingested. When disposed of through sink, flush with large quantity of water.
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 dessicant found saturated.
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 dessicant.
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 Antigen *P. falciparum* (HRP2) Card Test 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 pouch 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 immidiately 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



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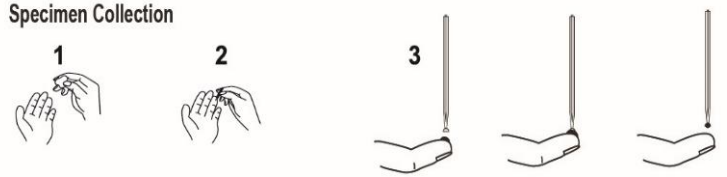
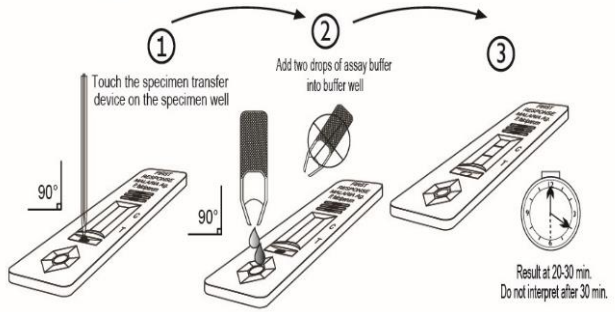
Specimen Collection , Test Procedure & Result Interpretation

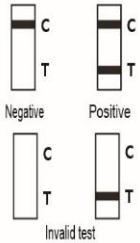
Specimen Collection



Test Procedure



Results Interpretation



The invalid test result (absense of control line)should be retested with new test device

Manufactured by

Premier Medical Corporation Private Limited

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Part No.: (S)PI13-INS-002, Rev.: AC

• ISO 13485 & EN ISO 13485 Certified Company

②