REF PI16FRC25CE & PI16FRC30CE CE IVD 1°C 40°C

First Response Malaria Ag. pLDH/HRP2 Combo Card Test

Rapid One Step Malaria Ag. pLDH/HRP2 Combo Test

A rapid test for the detection of Malaria pLDH and HRP2 in human blood for *in vitro* test use only.

Intended Use

First Response® Malaria Ag. pLDH/HRP2 Combo Card Test is intended to be performed by trained users and as *in vitro* 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 following anti-coagulants such as heparin, EDTA or citrate do not affect the test results. 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, anemia and is caused by a parasite that is transmitted from one human to another by the bite of infected *Anopheles* mosquitoes. There are four kinds of *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 and it infects 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 Ag. pLDH/HRP2 Combo Card Test is based on principle of immuno-chro-matography 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 Dehydro-genase (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, whicHeads 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.

Material Provided	PI16FRC25CE	PI16FRC30CE
Test Device Pouch Containing: 1 Test Device, 1 Desiccant,	25 Nos.	30 Nos.
Specimen Transfer Device	25 Nos.	30 Nos.
Assay Buffer Bottle	1 No.	1 No.
Sterile Single-use Lancets	25 Nos.	30 Nos.
Alcohol Swabs	25 Nos.	30 Nos.
Instructions for use	1 No.	1 No.

Materials Required but Not Provided

- New pair of disposable gloves
- Pen
- Timer
- Extra lancets and alcohol swabs, if needed
- Sharps box
- Non-sharps disposal container
- Venipuncture blood collection materials and precision pipette plus tips (if whole blood is collected by venipuncture)
- Bio-hazardous waste container

Storage & Stability

First Response® Malaria Ag. pLDH/HRP2 ComboCard Test should be stored at 1-40 °C. Do not freeze the kit or components. Assay Buffer (opened& unopened) & the unopened Test Device are stable until the expiry date printed on the label, when stored atroom temperature 1-40 °C. The test device is sensitive to humidity and heat. Perform the test immediately after removing the Test Device from the foil pouch. The shelf life of the kit is as indicated on the outer package. Do not use Test Device and Assay Buffer beyond the date of expiry.

Precautions

1) For *in vitro* diagnostic use only.
2) Test Devices and Assay Buffer of different lot must not be used.
3) Do not use the Test Device if the pouch is not intact.

4) Do not use the Sterile lancet if the seal is broken.
5) Check the desiccant for saturation, immediately after opening the pouch.
6) Do not smoke, eat or drink while handling specimens and performing a test.
7) The test device, alcohol swab, lancet and specimen transfer device are intended for single use only.
8) Follow the assay procedure strictly, deviation will invalidate the results.
9) Perform the test by using kit buffer, any other buffer or fluid will invalidate the results.
10) Do not touch the tip of Assay Buffer bottle, it might contaminate buffer.
11) Wear protective gloves while handling specimens. Dispose of used gloves as biohazard waste. Wash hands thoroughly afterwards.
12) Avoid splashing or aerosol formation.
13) Clean up spills thoroughly using an appropriate disinfectant.
14) Decontaminate and dispose of all used specimens, test devices, alcohol swabs and Specimen transfer device as a infectious waste, in a biohazardous waste container. Dispose of used lancets in a sharps box.

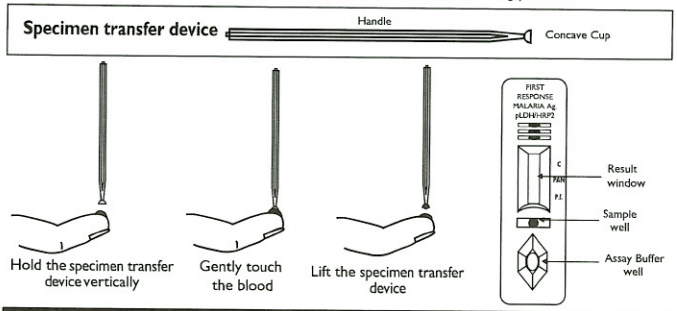
Specimen Collection and Storage

[Collection by venipuncture]

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

[Collection using a lancet]

1) Clean the area to be lanced with an alcohol swab.
2) Squeeze the end of the fingertip and pierce with a sterile lancet provided.
3) Wipe away the first drop of blood with gauze or cotton.
4) Take an specimen transfer device(Inverted Cup)provided. Hold it vertically Gently touch the open concave end into the blood. as shown in following picture.

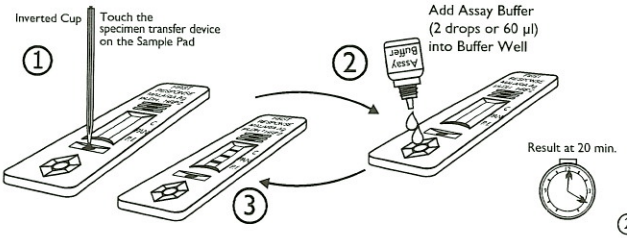


Test Procedure

Ensure that the Device and buffer are at room temperature before starting the procedure.

1) Take the Test Device and the Specimen transfer Device from the kit. Label the Test Device with the patient identification number / name. Place the test device on a flat surface.
2) Transfer 5µl of whole blood collected in an Specimen transfer device into Sample well by touching Sample Pad.
3) Add two drops (60 µl) of Assay Buffer into the Assay Buffer Well.
4) Read & interpret the test results at 20 minutes after the addition of specimen & buffer to test device. Do not read after 30 minutes.

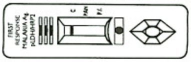
Note : The test result for some strongly reactive specimens may become visible sooner than 5 minutes however, it is always advised to wait for minimum 20 minutes before interpreting the test result.



Interpretation of the Test

Non-reactive Result

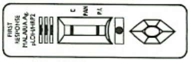
If only one color band appear, at control line 'C' as in the figure, the specimen is negative.



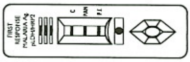
Negative

Reactive Results

If two color bands appears, one at control line 'C' and the other at test line 'P.f.', indicates the specimen is Positive for *P. falciparum* (in case of low parasitemia). If all three color bands appears, one at control line 'C', and the other two at 'P.f.' and 'PAN' as in the figure, it indicate specimen is Positive for either *P. falciparum* or mix infection of *P. falciparum* with any of other PAN malaria species.



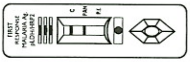
OR



P. falciparum ***P. falciparum* or mixed infection**

If two color bands appears, one at control line 'C' and other at test line 'PAN' as in the figure, the specimen is *P. vivax, P. malariae* or *P. ovale* positive.

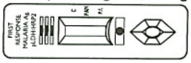
Interprete faint line as reactive line.

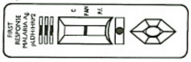


PAN Positive

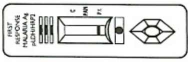
Invalid Result

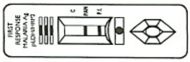
If no color band appear, at control line 'C' within the stipulated time then the result is invalid. The result is also invalid if a color band appears only at test line 'Pf / PAN / Pf & PAN'. If high background and incomplete imigration along the test strip then the results is invalid.





Invalid





Performance Characteristics

(A) 1. The Performance of First Response® Malaria Ag. pLDH/HRP2 Combo Card Test was evaluated using an in-house panel of Positive and Negative Malaria specimens. The Status of the specimens was determined by microscopic examination. The results are as follows :

Specimens	No. of Specimens Tested	FIRST RESPONSE® Malaria Ag. pLDH/HRP2 Combo Card Test		Sensitivity (95% confidence intervals)	Specificity (95% confidence intervals)
		Positive	Negative		
P. falciparum Positive	224	224	0	100% (97.89% - 100%)	-
P. vivax Positive	234	234	0	100% (97.98% - 100%)	-
Malaria Negative	964	0	964	-	100% (99.50% - 100%)

(A) 2. The Performance of First Response® Malaria Ag. pLDH/HRP2 Combo Card Test was evaluated at various external laborato ries & institut es.The summary of reports are as follows :

Name of the Institute	Year of Testing	Sensitivity				Specificity
		200 parasite/µl		2000 parasite/µl		
		P.f. Sample	*P.v.* Sample	*P.f.* Sample	*P.v.* Sample	
Results of WHO product testing of malaria RDTs: round 6	2014-15	82%	91.4%	100%	100%	98.1%

B) Analytical sensitivity :- The sensitivity of First Response® Malaria Ag. pLDH/HRP2 Combo Card Test for*P. falciparum* and PAN Malarial Species is comparable to microscopic observation with more than or equal to 200 parasites per µL of blood. The product has not been fully assessed for *P. ovale* & *P. malarie* and the sensitivity is expected to be low (<50%).

Precision

Within-run and between-run precisions have been determined by testing of 10 replicates of three specimens: a negative, a low positive and a strong positive. The agreement between the test results and the expected results were 100%.

Limitations and Interferences

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: hesparin, EDTA & 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 pLDH & 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/µl), very high parasite density (prozone/hook effect), mutations in the HRP-2 gene with deletion of HRP-2 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.
9) Repeat after 8 hours if suspicion of malaria persists.

Note: Malaria RDTs can give positive results after successful treatment. This is particularly the case for HRP-2, which can remain in the blood for weeks. Therefore, malaria RDTs are not recommended for monitoring treatment of malaria.

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	EC REP	Representative Authorised

References

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- ISO 13485:2003 Certified Company
- EN ISO 13485:2012 Certified Company

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