WHO Prequalification of In Vitro Diagnostics Programme
PUBLIC REPORT

Product: ParaHIT f Ver. 1.0 Rapid Test for P.falciparum Malaria Device
Number: PQDx 0062-023-01

Abstract

ParaHIT f Ver. 1.0 Rapid Test for P.falciparum Malaria Device with product codes **55IC104-10, 55IC104-25, 55IC104-50** manufactured by Arkray Healthcare Private Limited., **CE-marked regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 7 October 2014. This WHO prequalification public report was amended on 2 March 2016.

Intended use:
ParaHIT f Ver. 1.0 Rapid Test for P.falciparum Malaria Deviceis an in vitro qualitative screening test for the detection of Plasmodium falciparum specific HRP – II (Histidine-rich protein II) in human blood.

Test principle:
The test is based on the principle of immunochromatography in which nitrocellulose membrane is coated with Anti-HRP-II antibody (capture antibody) which is specific for P. falciparum. When the test sample along with Reaction Buffer flows through the conjugate pad, the colloidal gold coupled with Anti-HRP-II antibodies (detection antibody) binds to HRP-II antigens. HRP-II antigen is released from the lysed infected red blood cells of test sample. This antigen-detector antibody complex moves along the nitrocellulose membrane and binds to the corresponding immobilised antibodies to HRP-II(capture antibody) leading to the formation of red colour band which indicates reactive results. The control band should appear irrespective of reactive or non-reactive sample. The visualisation of control band indicates successful migration of the reaction mixture.

The test kit contains:
55IC104-10 (10 tests): Includes 10 test devices, 10 specimen transfer devices (single use, disposable plastic pipettes), 1 bottle of reaction buffer (2.5 ml), 10 lancets, 10 alcohol swabs, and 1 instruction for use (IFU).

55IC104-25 (25 tests): Includes 25 test devices, 25 specimen transfer devices (single use, disposable plastic pipettes), 1 bottle of reaction buffer (6 ml), 25 lancets, 25 alcohol swabs, and 1 instruction for use (IFU).

55IC104-50 (50 tests): Includes 50 test devices, 50 specimen transfer devices (single use, disposable plastic pipettes), 1 bottle of reaction buffer (12 ml), 50 lancets, 50 alcohol swabs, and 1 IFU.

Storage:
The test kit should be stored at 4 °C to 40 °C.

Shelf-life:
24 months.

Summary of prequalification status for ParaHIT f Ver. 1.0 Rapid Test for P. falciparum Malaria Device

	Initial acceptance	
	Date	**Outcome**
Amended PQ	2 March 2016	listed
Status on PQ list	7 October 2014	listed
Dossier assessment	28 March 2014	MR
Inspection status	30 June 2014	MR
Laboratory evaluation	2011	MR

MR: Meets Requirements
NA: Not Applicable

ParaHIT f Ver. 1.0 Rapid Test for P.falciparum Malaria Device was accepted for the WHO list of in vitro prequalified diagnostics on the basis of data submitted and publicly available information.

Background information

Arkray Healthcare Private Limited submitted an application for prequalification of ParaHIT f Ver. 1.0 Rapid Test for P.falciparum Malaria Device. Based on the results of the WHO product testing of malaria RDTs Round 3, ParaHIT f Ver. 1.0 Rapid Test for P.falciparum Malaria Devicewas given priority for prequalification.

Product dossier assessment

Arkray Healthcare Private Limited submitted a product dossier for ParaHIT f Ver. 1.0 Rapid Test for P.falciparum Malaria Deviceas per the *Instructions for compilation of a product dossier* (PQDx_018 v1). The information submitted in the product dossier was reviewed by

WHO staff and external experts (assessors) appointed by WHO in accordance with the *internal report on the screening and assessment of a product dossier* (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for ParaHIT f Ver. 1.0 Rapid Test for P.falciparum Malaria Device for prequalification.

The manufacturer committed to amend and submit additional documentation on the following issues which will be reviewed at the next re-inspection:

1. Final real time studies report supporting stability claims.
2. Minor amendments to the Instructions for Use.

Manufacturing site inspection

A comprehensive inspection was performed at the manufacturing site (Plot no 336/338/340, Road no 3, G.I.D.C., Sachin (Surat), 394230, India) of the ParaHIT f Ver. 1.0 Rapid Test for P.falciparum Malaria Devicetest in March 2014 as per the *Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics* (PQDx_014 v1). 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 5 September2014.

Laboratory evaluation

The third round of WHO product testing of RDTs for malaria antigen detection was completed in 2011 under the name ParaHIT-f (Device).

The product was evaluated against a *Plasmodium falciparum* cultured line panel, *Plasmodium falciparum* wild type parasite panel and a *Plasmodium falciparum* 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.

The panel detection score using *P. falciparum* was 84.9%, the false-positive rate was 0% and invalid rate for *P. falciparum* was 0%.

Therefore ParaHIT f Ver. 1.0 Rapid Test for P. falciparum Malaria Device meets the current laboratory evaluation requirements for prequalification.

Change notification

In 2015, M/S Span Diagnostics Ltd., submitted a change notification related change of ownership of the manufacturer to Arkray Healthcare Private Limited. This change notification was assessed and product was found to meet WHO prequalification requirements.

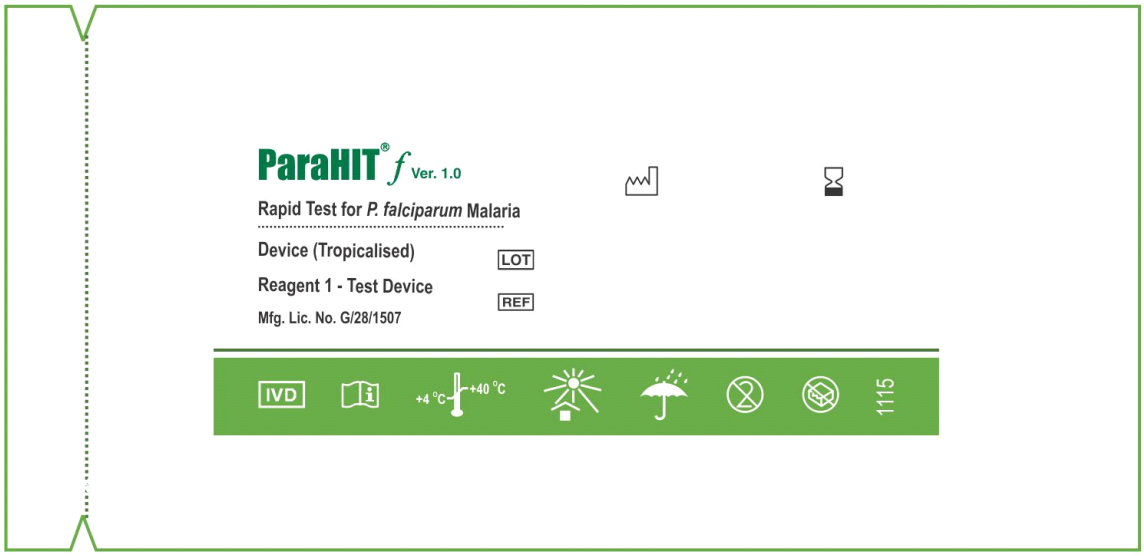
Labelling

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

1. Labels

1.1 Label for Test Device pouch

Front Side



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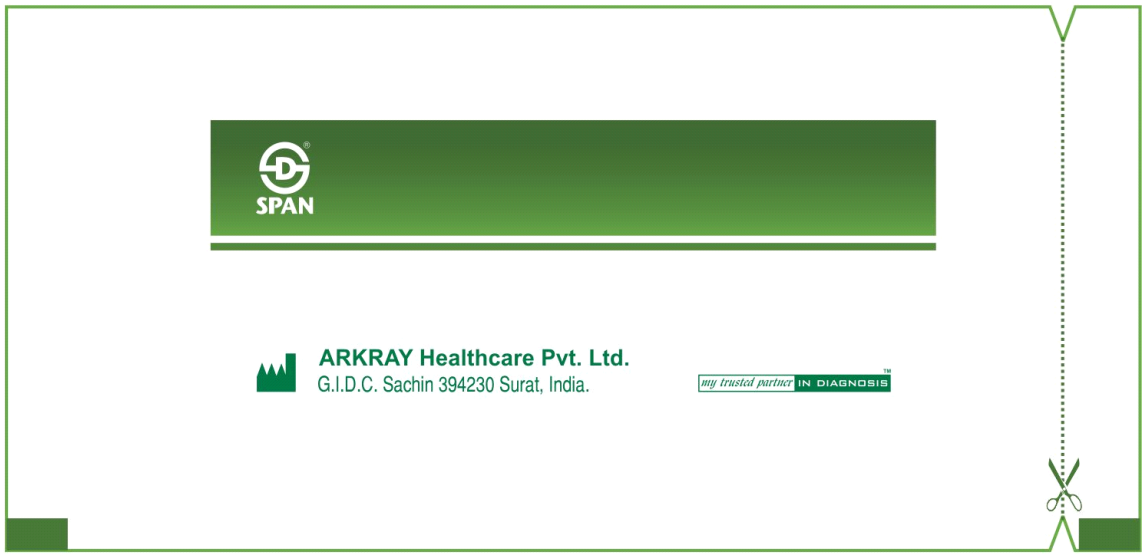

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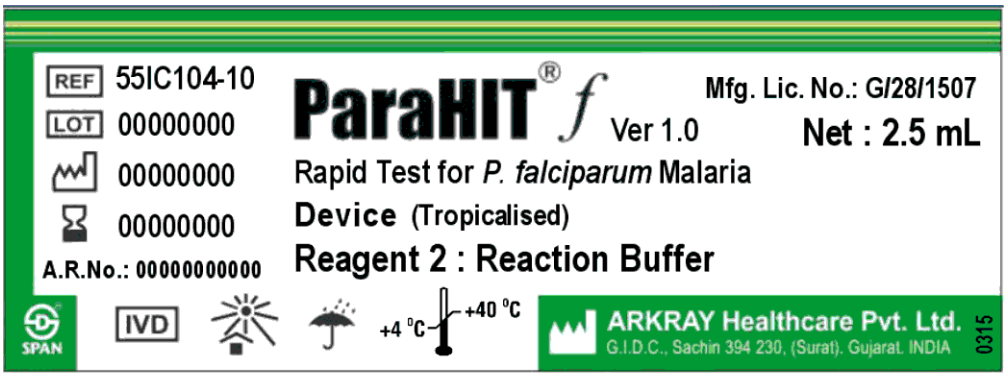
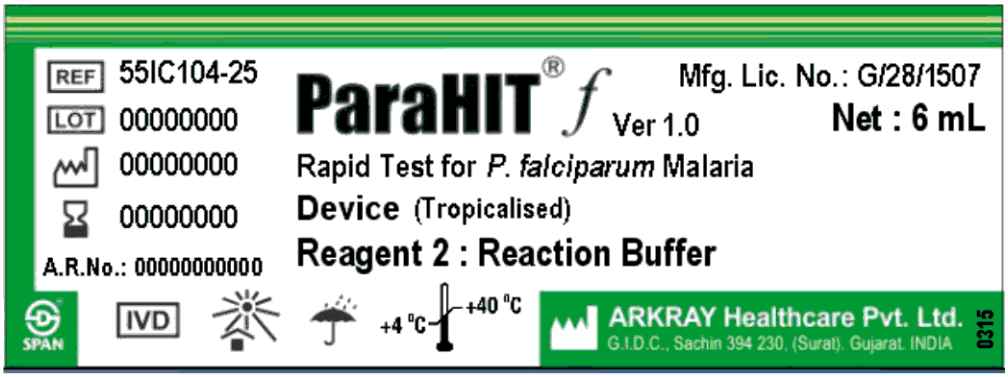
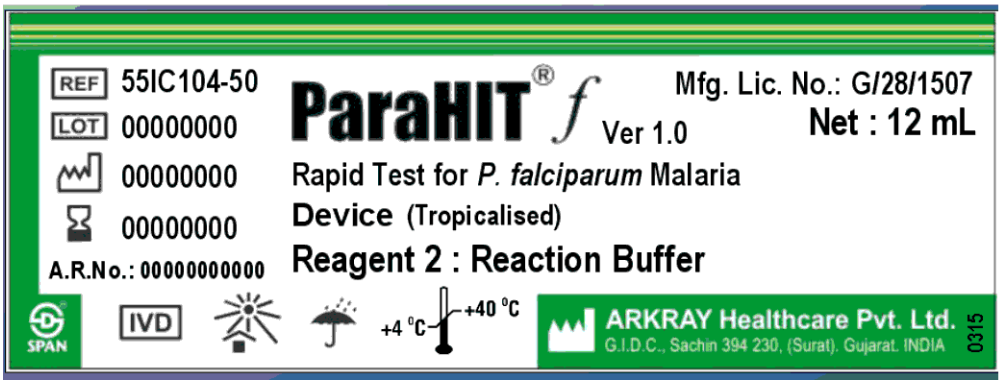
1.2 Labels for Reaction Buffer (55IC104-10, 55IC104-25 & 55IC104-50)







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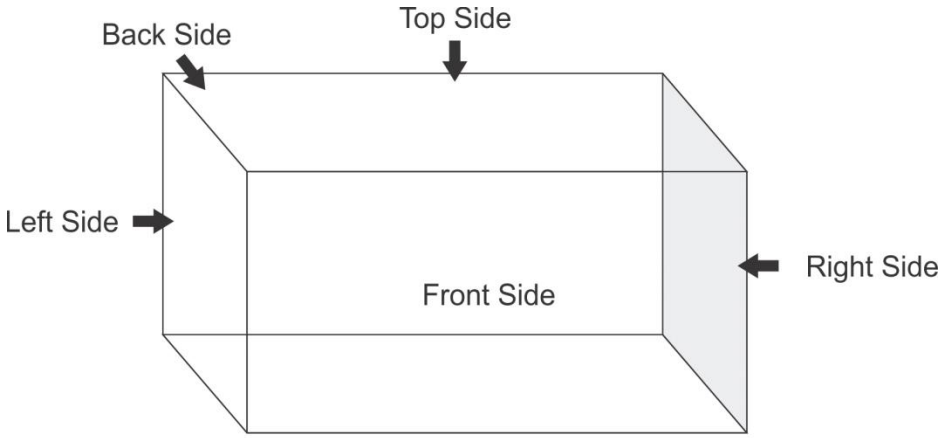

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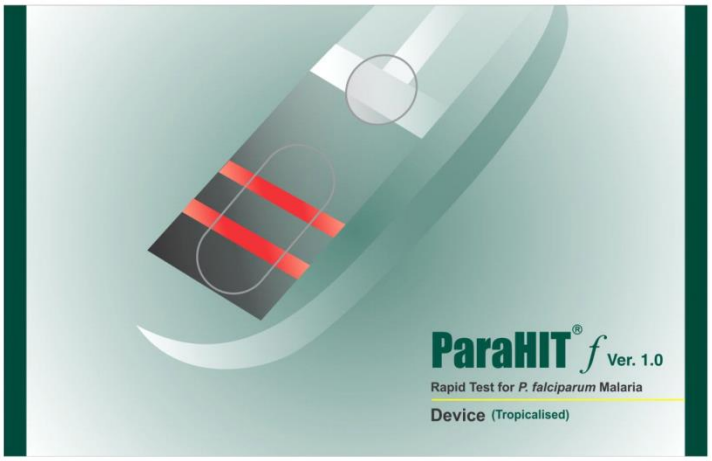

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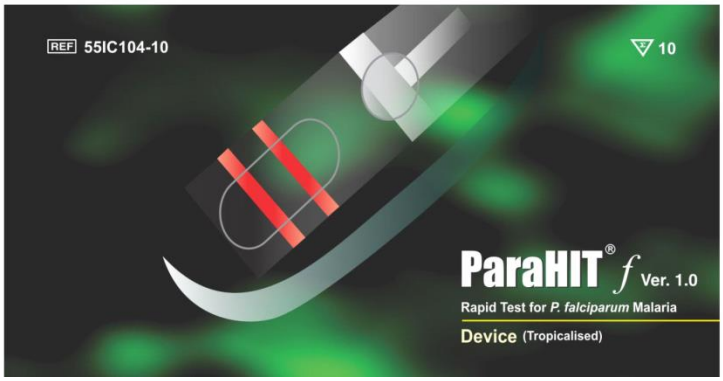

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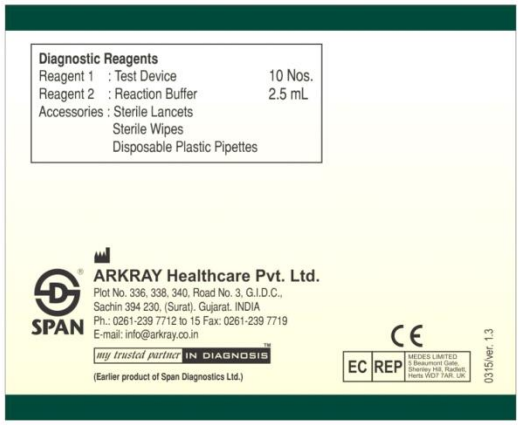

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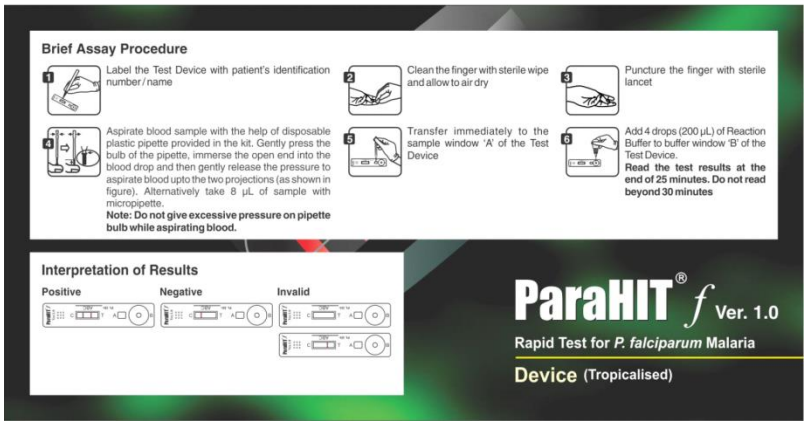

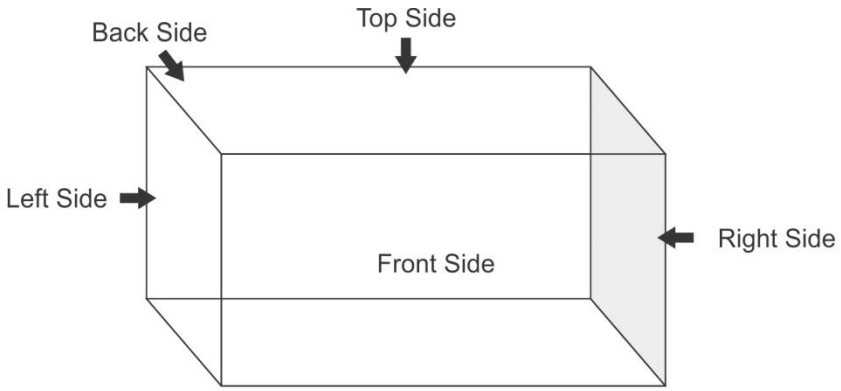

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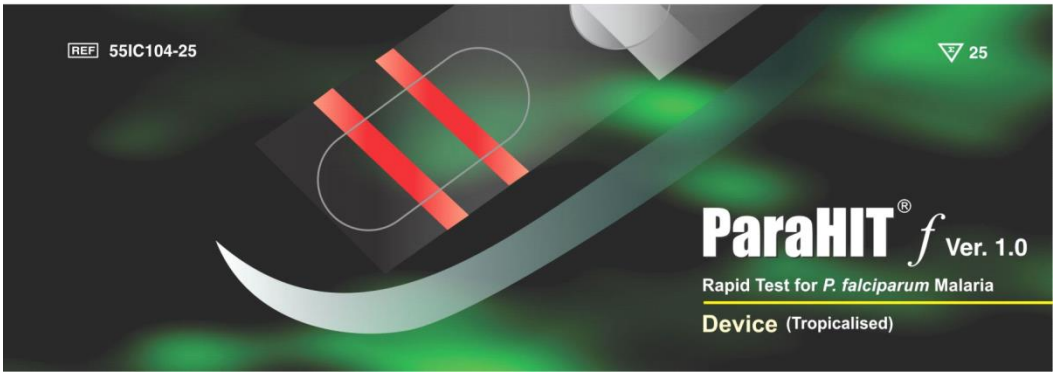

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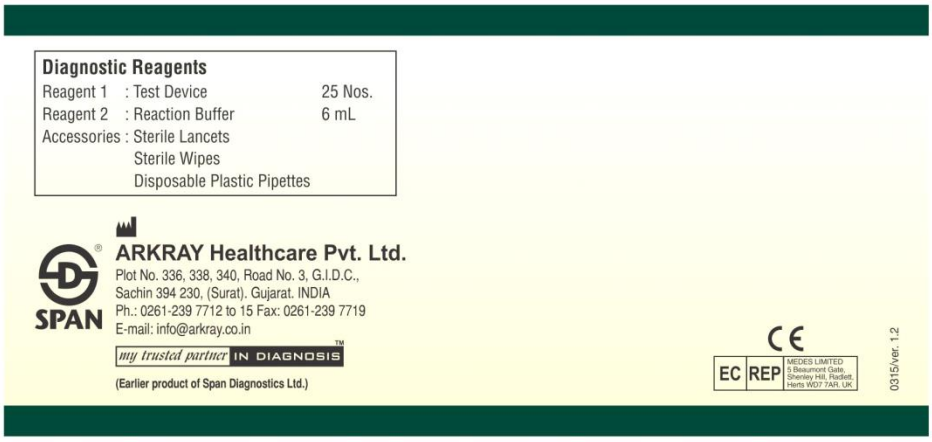

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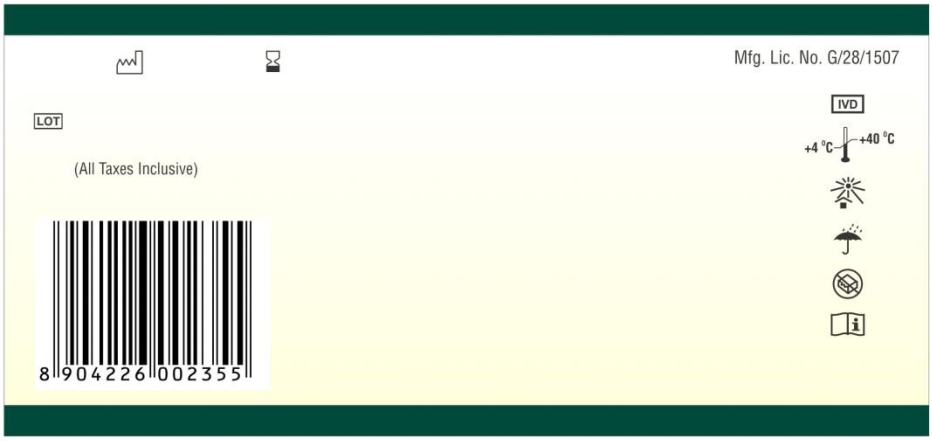

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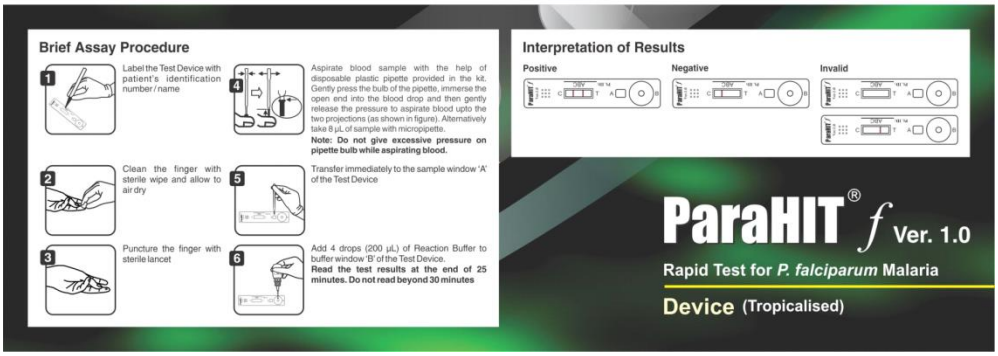

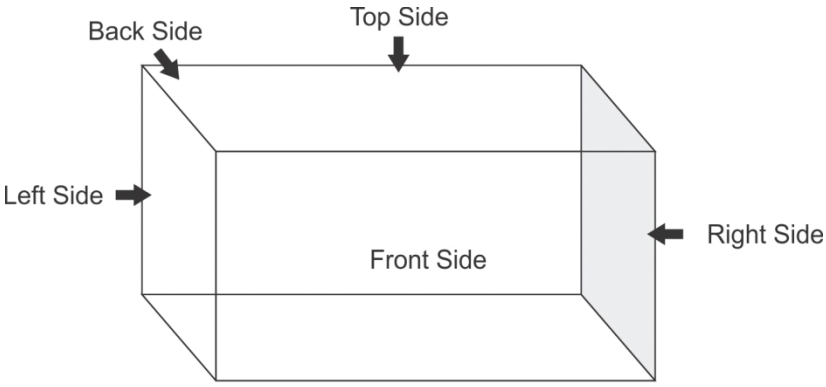

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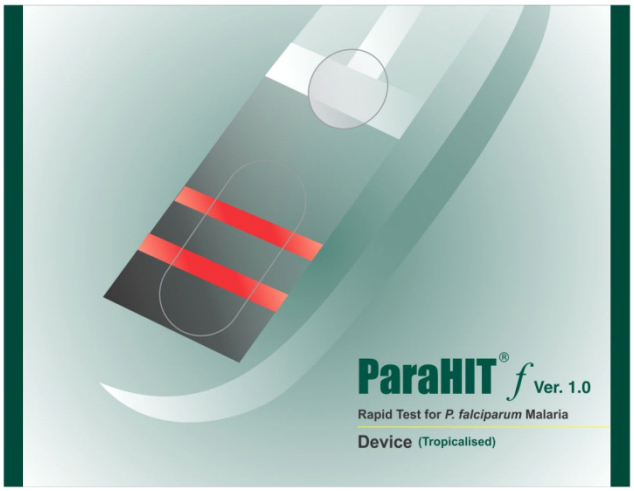

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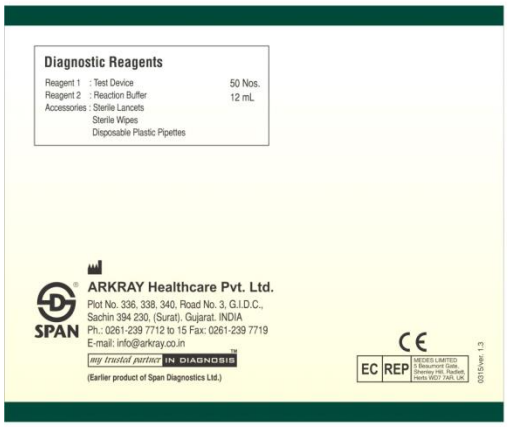

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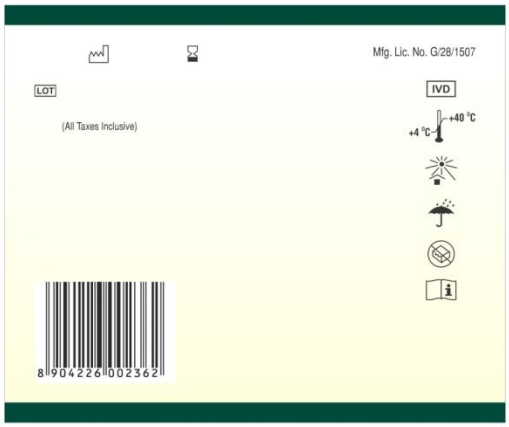

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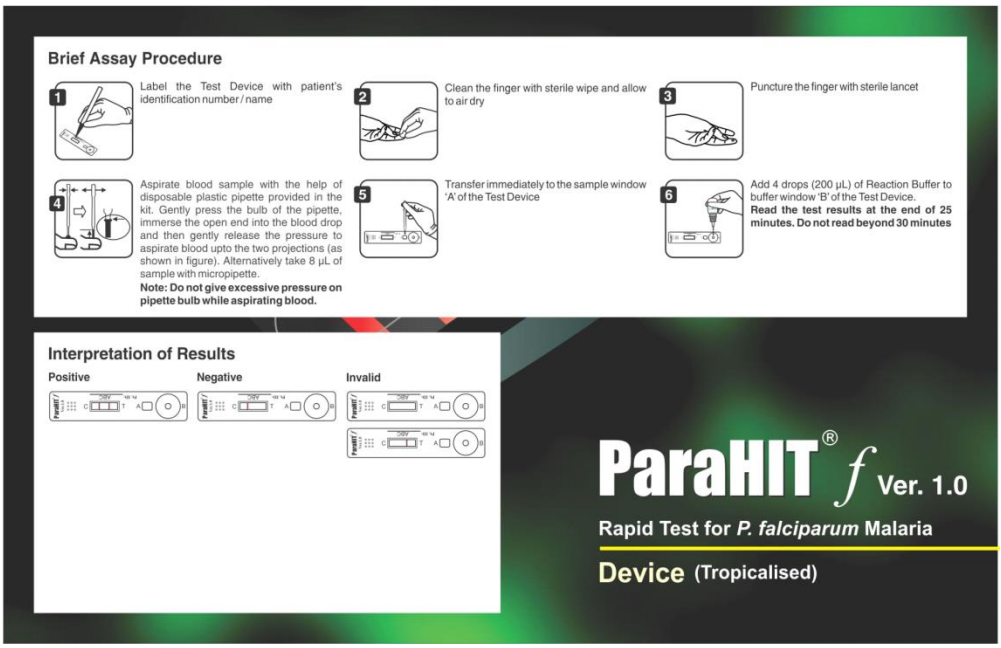


2. Instructions for use

ParaHIT® *f* Ver. 1.0

IVD +4 °C +40 °C

Rapid Test for *P. falciparum* Malaria - Device

REF 55IC104-10, 55IC104-25, 55IC104-50

Σ 10, 25, 50

INTENDED USE

ParaHIT® *f* Ver. 1.0 - Rapid Test for *P. falciparum* Malaria provides a simple, rapid and an *in vitro* qualitative screening test for the detection of *Plasmodium falciparum* specific HRP II (histidine rich protein II) in human blood. It does not require additional instrument.

INTRODUCTION

ParaHIT® *f* Ver. 1.0 - Rapid Test for *P. falciparum* Malaria is for the detection of Pf HRP II, a soluble protein which is induced by malarial parasites (*P. falciparum*) and released from infected red blood cells[1].

The assay is intended for use by Health Care Workers / Laboratory Professionals, only for an initial screening. The assay is to be used in the diagnosis of malaria in symptomatic patients.

This test kit is standardized to withstand tropical conditions.

PRINCIPLE

ParaHIT® *f* Ver. 1.0 - Rapid Test for *P. falciparum* Malaria is based on the principle of immunochromatography in which nitrocellulose membrane is coated with Anti-HRP II antibody (capture antibody) which is specific for *P. falciparum*.

When the test sample along with Reaction Buffer flows through the conjugate pad the colloidal gold coupled with Anti-HRP II antibodies (detection antibody) binds to HRP II antigen. HRP II antigen is released from the lysed infected red blood cells of test sample. This antigen-detector antibody complex moves along the nitrocellulose membrane and binds to the corresponding immobilised antibodies to HRP II (capture antibody) leading to formation of red colour band which indicates reactive results. The control band should appear irrespective of reactive or non-reactive sample. The visualisation of control band indicates successful migration of the reaction mixture.

KIT CONTENTS AND DESCRIPTION

REAGENTS

Reagent No.	Reagent Name	Description
Reagent 1	Test Device	Test Device comprising of nitrocellulose membrane with Anti-HRP II antibodies immobilised at 'T' region, control reagent immobilised at 'C' region and conjugate releasing pad impregnated with Anti-HRP II antibody colloidal gold conjugates.
Reagent 2	Reaction Buffer	Physiological buffer containing detergent and preservative.

ACCESSORIES

Sterile Lancets - Twist Head Needle Type

Sterile Wipes - 70% Isopropyl alcohol impregnated

Disposable Plastic Pipette

MATERIALS REQUIRED BUT NOT PROVIDED IN THE KIT

1. Timer
2. Biohazard Disposable Container
3. Sharp Container
4. Pen / Pencil
5. Disposable Gloves

STORAGE AND STABILITY

Store at +4 °C to +40 °C, away from direct sunlight and humidity. The Test Device (Reagent 1) is stable for two years only in unopened condition whereas Reaction Buffer (Reagent 2) is stable for two years in opened / unopened conditions. May be refrigerated. **Do not freeze**. Once opened the Test Device should be used immediately. The shelf life of the kit is as indicated on the outer package. **Do not use Test Device and Reaction Buffer beyond the date of expiry.**

SPECIMEN COLLECTION AND HANDLING

Specimen to be used: Whole blood (capillary or venous blood)

For capillary blood : Clean the skin thoroughly with sterile wipe (provided in the kit). Allow the area to air dry before collection of sample.

For venous blood: If venous blood is used, it should be collected with an anticoagulant such as Heparin or EDTA (Neither of these anticoagulants has been shown to interfere with test results). If the test can not be performed immediately, the blood may be stored up to 3 days at +2 °C to +8 °C. If blood is refrigerated, allow it to come to room temperature (+15 °C to +30 °C) prior to testing. Mix gently before testing. Using the specimen more than 3 days after collection can cause non-specific reaction.

BIOSAFETY

1. Handle all samples with care, as they can be potentially infectious.
2. Wear disposable gloves throughout the test procedure and dispose them of as biohazard waste.
3. Wear protective laboratory clothings in laboratory areas.
4. Do not smoke, eat or drink in area where samples are being handled.
5. Technicians with wound, cut or skin abrasions on the hand must refrain from performing the test without proper precautions.
6. Avoid spilling of samples or solutions containing samples. In case of spillage, immediately clean it with 1:10 dilution of 5% freshly prepared sodium hypochlorite solution and dispose of the cleaning material by a suitable method.
7. Decontaminate and dispose of alcohol swabs and lancets in a biohazard disposable container.
8. Dispose of silica gel packets by breaking the packets open and discarding the contents in the waste bins.
9. Wash hands thoroughly with disinfectant after completion of the test.

PRECAUTIONS

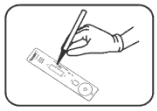
1. The kit should be used for *in vitro* diagnostic use only.
2. Protect the Test Device from humidity and heat.
3. Test Device and Reaction Buffer of different lot must not be used. Use of any buffer other than provided in the kit may give false result.
4. Do not use the Test Device if the pouch is not intact.
5. At the time of opening the pouch, if the desiccant colour has turned from blue to white do not use that test assembly.
6. Do not use the lancet if the seal is broken as it may lead to any infection at punctured skin due to cease in its existing sterility.
7. Follow the assay procedure strictly, deviation will invalidate the results.
8. Do not use any other specimen than whole blood.
9. Caution should be taken while interpreting results with potentially interfering samples like hemolytic samples, Rheumatoid Factor containing samples etc.
10. All disposable plastic pipette, sterile lancet & Test Device are single use items, do not use with multiple specimens. Contamination of dispensing equipment, containers or reagents can lead to inaccurate results.

PROCEDURE

ASSAY SETUP

Ensure that the Test Device and Reaction Buffer are at room temperature (+15 °C to +30 °C) before starting the assay procedure.

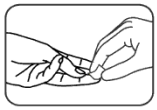
1. To open the leak proof dropper Reaction Buffer bottle, tighten the cap further to pierce the top of the nozzle of bottle and then remove the cap to deliver the drop.
2. Remove the Test Device from pouch and label with the patient's identification number / name (as shown in figure).



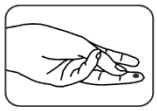
Note: Dispose of silica gel packets as mentioned in bio-safety point no. 8.

ASSAY PROCEDURE

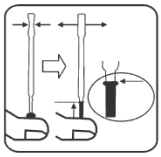
1. Select the finger for puncture, clean with sterile wipe and allow to air dry completely.



2. Puncture the finger with sterile lancet provided in the kit.

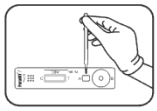


3. Aspirate blood sample with the help of disposable plastic pipette provided in the kit. Gently press the bulb of the pipette, immerse the open end into the blood drop and then gently release the pressure to aspirate blood upto the two projections (as shown in figure). Alternatively take 8 µL of sample with micropipette and transfer immediately to the sample window 'A' of the Test Device.

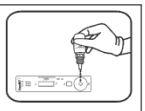


Note: Do not give excessive pressure on pipette bulb while aspirating blood.

Note : In case of venous whole blood, use the disposable plastic pipette or micropipette for taking required quantity of blood and transferring to the Test Device.



4. Add 4 drops (200 µL) of Reaction Buffer to buffer window 'B' of the Test Device.



Note: After adding each drop allow 10 seconds for soaking.

5. Read the test results at the end of 25 minutes. Do not read beyond 30 minutes. READING TOO LATE CAN GIVE FALSE RESULTS.

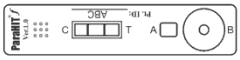
After reading the results dispose of the Test Device & disposable plastic pipette as biohazard waste.

INTERPRETATION OF RESULTS

POSITIVE

Pf Positive

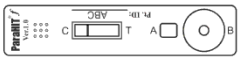
Appearance of two red coloured bands each at test (T) & control (C) region indicate the presence of *P. falciparum* antigen (HRP II).



Note: Test line of any intensity (light to dark) should be considered positive.

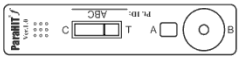
NEGATIVE

Appearance of single red coloured band at control (C) region indicates the absence of *P. falciparum* antigen (HRP II).



INVALID

Absence of red coloured band at control (C) region, with or without band at the test (T) region indicates invalid results. Repeat the test using a fresh Test Device.



Note : The control band should appear irrespective of reactive or non-reactive sample. The visualisation of control band indicates successful migration of the reaction mixture. Therefore, the colour of control band should not be compared with colour of test band for interpretation of results.

DISPOSAL OF ASSAY

Remnants of samples, used Test Device and buffer should be discarded as biohazard waste through disinfection and followed by incineration.

PERFORMANCE CHARACTERISTICS[3]

A SENSITIVITY AND SPECIFICITY

The sensitivity and specificity for **ParaHIT® f Ver. 1.0** - Rapid Test for *P. falciparum* Malaria is 99.42% and 99.59% respectively. The performance of test was established by comparison with the results of microscopic examination of thick and thin films.

Result of ParaHIT® f Ver. 1.0	Reference Method (microscopic examination)		Total
	Positive	Negative	
Positive	172	4	176
Negative	1	977	978
Total	173	981	1154

% Specificity	% CI
99.59	99.2-100

% Sensitivity	% CI
99.42	98.3-100

B ANALYTICAL SENSITIVITY (Limit of detection)

The sensitivity of **ParaHIT® f Ver. 1.0** - Rapid Test for *P. falciparum* Malaria is comparable to microscopic observation with more than or equal to 100 parasites per µL.

C ANALYTICAL SPECIFICITY (Cross reactivity)

The **ParaHIT® f Ver. 1.0** - Rapid Test for *P. falciparum* Malaria does not cross react with other species of malaria; *viz. P. malariae, P. ovale,* and *P. vivax.*

D PRECISION

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

E RESIDUAL RISK : None

LIMITATIONS OF THE TEST

1. The test procedure, precautions and interpretation of results for this test must be followed when testing.
2. Final diagnosis should not be made until all clinical as well as laboratory findings have been evaluated.
3. Less than 100 parasites / µL may give false negative results.
4. This test kit can provide fast and easy way to get a result, but do not completely exclude the possibility of false positive or false negative results caused by various factors.
5. Interfering samples like hemolytic (hemoglobin containing) samples, icteric (bilirubin containing) samples, lipaemic samples and Rheumatoid Factor (RF) containing samples may affect the test results[3].
6. A positive test should be carefully interpreted to distinguish between a new infection and effectively treated old infections. This is due to the persistence of HRP II antigen in the blood for 1-3 weeks after effective treatment.[2]
7. Any modification to the above procedure and / or use of other reagents should invalidate the test procedure.
8. In case of strong clinical evidence of Malaria a negative test should be repeated.
9. This assay cannot be used for the diagnosis of infection by other type of malarial parasites (*P. malariae, P. vivax, P. ovale* or *P. knowlesi*).

PRESENTATION

Reagent No.	Reagent Name	REF 55IC104-10 / Σ 10	55IC104-25 25	55IC104-50 50
Reagent 1	Test Device	10	25	50
Reagent 2	Reaction Buffer	2.5 mL	6 mL	12 mL
Accessories	Sterile Lancets Sterile Wipes Disposable Plastic Pipettes	10 10 10	25 25 25	50 50 50

REFERENCES

1. Howard R. J., et al.,1986 : Secretion of a Malarial Histidine Rich Protein (PfHRP II) from *Plasmodium falciparum* infected Erythrocytes. *J.Cell. Biol.* **103**, 1269-1277.
2. WHO: Guidelines for Treatment of Malaria. Second Edition (2010) Annex 5 Malaria Diagnosis, 119.
3. Data on internal file.

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SYMBOL LEGENDS

Symbol	Explanation of Symbol	Symbol	Explanation of Symbol
	Consult instructions for use	Σ	Contains sufficient for 'n' tests
	Do not use if package is damaged	REF	Catalogue number
IVD	*In vitro* diagnostic device	LOT	Batch code No.
+4°C +40°C	Store at +4 °C to +40 °C		Manufacturer
	Keep away from sunlight		Date of manufacture
	Keep dry		Use by (date or month of expiry)
	Do not reuse	EC REP	Authorized Representative

LIMITED EXPRESSED WARRANTY DISCLOSURE

ARKRAY Healthcare Pvt. Ltd. (ARKRAY) limits the warranty to the test kit in as much as the said test kit will function only within the limitations and specifications as described and illustrated in the product insert. Any deviation there from by the purchaser or the end user shall not be the liability and/or responsibility of ARKRAY. ARKRAY shall not be liable and / or responsible for any misuse of the said test kit after the date of expiry. If any defect is proved in the manufacture of the test kit, ARKRAY shall be liable only to the extent of the replacement of the said test kit or the refund of its purchase price thereof and shall not be liable for any consequential loss arising there from.

SPAN

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