WHO Prequalification of In Vitro Diagnostics Programme
PUBLIC REPORT

Product: ParaHIT f Ver. 1.0 Rapid Test for P.falciparum Malaria Device

WHO Reference Number: PQDx 0062-023-00

ParaHITf Ver. 1.0 Rapid Test for P.falciparum Malaria Device with product codes **55IC104-10, 55IC104-25, 55IC104-50, 55IC116-10, 55IC116-25, and 55IC116-50** 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.

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

	Date	Outcome
Prequalification listing	7 October 2014	listed
Dossier assessment	28 March 2014	MR
Site inspection(s) of quality management system	18 September 2024	MR
Product performance evaluation	2011	MR

MR: Meets Requirements

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

Version	Summary of amendments	Date of report amendment
2.0	Change of ownership of the manufacturer to Arkray Healthcare Private Limited.	2015
3.0	Revisions to the labelling and packaging.	2017
4.0	Introduction of the Disposable Inverted Cup as a Sample Transfer Device as part of new configurations with product codes 55IC116-10, 55IC116-25, and 55IC116-50.	5 August 2024.
5.0	Changed the Silica Gel bag from 1 gram to 0.5 gram.	26 June 2025

Intended use:

According to the intended use from Arkray Healthcare Private Limited, "*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 specific HRP II (histidine rich protein II) in human blood. It does not require additional instrument.*

The assay is intended for use by Health Care Workers / Laboratory Professionals, for an initial screening for malarial infection in symptomatic patients."

Test principle:

According to the intended use from Arkray Healthcare Private Limited, *"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 the Reaction Buffer, flows through the conjugate pad, the colloidal gold coupled with Anti-HRP-II antibodies (detection antibodies) 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 the 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."*

Test kit contents:

Component	Product code 55IC104-10 (10 Tests/kit (T/k))	Product code 55IC104-25 (25 T/k)	Product code 55IC104-50 (50 T/kit)	Product code 55IC116-10 (10 T/k)	Product code 55IC116-25 (25 T/k)	Product code 55IC116-50 (50 T/k)
Test device	10	25	50	10	25	50
Single-use, disposable plastic pipette	10	25	50	x	x	x
Single-use, disposable inverted cup	x	x	x	10	25	50
Reaction buffer	1 x 2.5 mL bottle	1 x 6 mL bottle	1 x 12 mL bottle	1 x 2.5 mL bottle	1 x 6 mL bottle	1 x 12 mL bottle
Sterile lancets	10	25	50	10	25	50
Alcohol swabs	10	25	50	10	25	50
Instructions for use	1	1	1	1	1	1

Materials required but not provided:

- Timer
- Biohazard disposable container
- Sharps container
- Pen/pencil
- Disposable gloves.

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

Shelf-life upon manufacture:
24 months.

Warnings/Limitations

Please refer to the current version of the Manufacturer's IFU for Warning/Limitations.

Prioritization for Prequalification

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. 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 Device was given priority for prequalification.

Dossier assessment

Arkray Healthcare Private Limited submitted a product dossier for ParaHITfVer. 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 ParaHITf Ver. 1.0 Rapid Test for P.falciparum Malaria Device for prequalification.

Manufacturing site inspection

At the time of considering the product application for Prequalification, the Manufacturer of the product had a well-established quality management system and manufacturing practices in place that would support the manufacture of a product of consistent quality.

Routine inspections of the Manufacturing site will be conducted with copies of the WHO Public Inspection Report (WHOPIR) published on the WHO Prequalification web page as per Resolution WHA57.14 of the World Health Assembly. Note that a WHOPIR reflects the information on the most current assessment performed at a manufacturing site for in vitro diagnostic products and summarises the assessment findings.

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

All published WHOPIRs are with the agreement of the manufacturer.
Based on the site inspection and corrective action plan review, the quality management system for the ParaHIT f Ver. 1.0 Rapid Test for P.falciparum Malaria Device meets WHO prequalification requirements.

Product performance 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 ParaHITf Ver. 1.0 Rapid Test for P. falciparum Malaria Device meets the current laboratory evaluation requirements for prequalification.

Labelling

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

1. Labels

1.1 Labels for product codes 55IC104-10, 55IC104-25 & 55IC104-50)

1.1.1 Packaging box artwork

1.1.2 Label for Test Device Pouch

1.1.3 Labels for Reaction Buffer

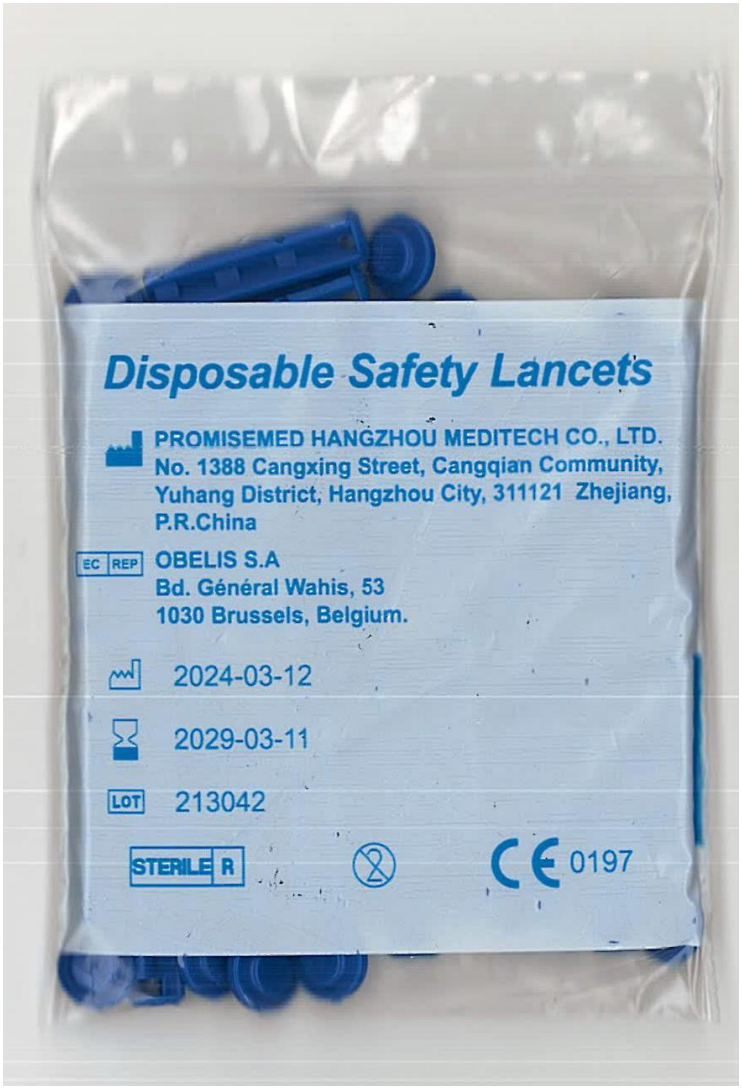
1.2 Labels for product codes 55IC116-10, 55IC116-25 & 55IC116-50

1.2.1 Packaging box artwork

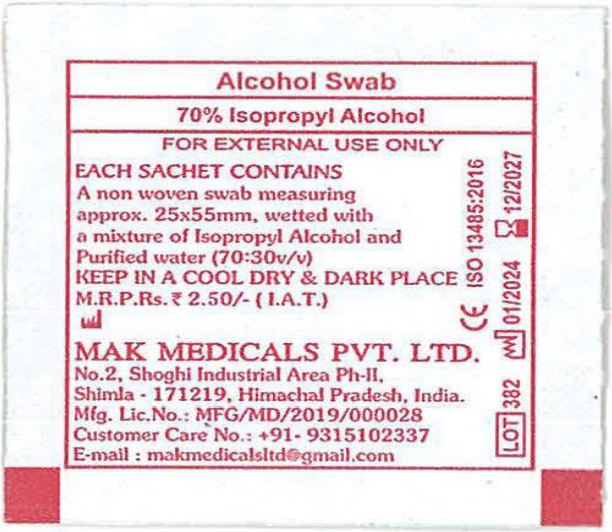
1.2.2 Label for Test Device Pouch

1.2.3 Labels for Reaction Buffer

1.3 Lancet label



1.4 Alcohol swab label



1.5 Desiccant label

To,
ARKRAY HEALTHCARE PVT LTD.
PLOT 336-338-340 , ROAD NO-3
G .I. D. C SACHIN . (SACHIN)

PRODUCT : 0.5GMOBN (COBALT FREE)
QUANTITY : 500 nos * 1 Pkt = 500 Nos
BATCH NO : AP30092022
CHALLAN No : NA
PO NO : FREE SAMPES
BOX no : 1 From,
ANIL PRODUCTS.
Navasari. Gujarat

Instructions for use[1]

[1] English version of the IFU was the one that WHO assessed. The manufacturer is responsible for ensuring correct translation into other languages.

ParaHIT® *f* ver. 1.0

Rapid Test for *P. falciparum* Malaria - Device

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

Σ : 10, 25, 50, 25

IVD +4 °C +40 °C

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.

The assay is intended for use by Health Care Workers / Laboratory Professionals, for an initial screening for malarial infection in symptomatic patients.

INTRODUCTION

Malaria is a life-threatening disease characterized by fever, chills, and anaemia and is caused by a parasite that is transmitted from one human to another by the bite of infected female *Anopheles* mosquitoes. There are five types of parasite species that can infect human : *Plasmodium falciparum*, *P. vivax, P. ovale, P. malariae* and *P. knowlesi*. Of these *P. falciparum* and *P. vivax*, pose the greatest threat. In 2015, 95 countries and territories had ongoing malaria transmission. Globally about 3.2 billion people are at risk of malaria.[1]

Diagnosis of malaia depends on detection of parasites microscopically or detection of antigens derived from malarial parasites by immunological techniques. **ParaHIT® *f* Ver. 1.0 -** Rapid Test for *P. falciparum* malaria is designed 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[2]. 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) 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 antibody (detector antibody) binds to HRP II antigen which 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 antibody to HRP II (capture antibody) leading to formation of red coloured 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 (*QS - Quantity Sufficient)

Sterile Lancets (Twist Head Needle Type)

Sterile Wipes (70% Isopropyl alcohol impregnated)

Disposable Plastic Pipettes

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 until expiry in unopened condition whereas Reaction Buffer (Reagent 2) is stable until expiry in opened / unopened conditions. May be refrigerated. Do not freeze and protect the kit from direct sunlight. The shelf life of the kit is as indicated on the outer package.

BIOSAFETY

1. Handle all samples with care, as they can be potentially infectious.
2. Wear disposable gloves throughout the test procedure and dispose them off as biohazard waste.
3. Wear protective laboratory clothing in laboratory area.
4. Technicians with wound, cut or skin abrasions on the hand must refrain from performing the test without proper precautions like covering the wounds while working with samples.
5. Prevent splashing or spilling of samples or solutions containing samples. In case of spillage, immediately clean it with 1:10 dilution of freshly prepared 5% sodium hypochlorite solution and dispose off the cleaning material by a suitable method.
6. Any accessory which comes in direct contact with the specimen and used Test Devices should be considered as contaminated material and should be treated appropriately as per laboratory procedure.
7. Wash hands thoroughly with disinfectant after completion of the test.
8. After reading the results dispose off the Test Device & disposable plastic pipette as biohazard waste.

SPECIMEN COLLECTION AND HANDLING

Specimen	Storage at	Stability	Remarks
Whole blood (Capillary /Venous)	+2 °C to +8 °C	Short term (Upto 3 days)	If blood is refrigerated, allow it to come to room temperature prior to testing. Mix gently before testing. Using the specimen more than 3 days after collection can cause non-specific reaction. The test performs well with frozen sample if it is thawed only once.
	-20 °C or lower	Long term	

PRECAUTIONS

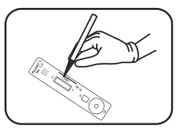
1. Do not use any specimen other than whole blood.
2. Do not use the reagents if they are unsealed or leaked or if the reagent bottle is not intact.
3. Do not use reagents after expiry date.
4. Do not combine the reagents from different lots of kits as they are optimized for individual lot to give best results.
5. Do not use the reagents, if they appear turbid or discoloured.
6. Open the sealed pouch at the time of assay performance only after it attains the room temperature.
7. Do not use the Test Device if the pouch is not intact.
8. On opening the pouch if the desiccant (silica gel) colour has changed to white, do not use that Device. Desiccant should be discarded as per local rules and regulations.
9. Once opened the Test Device should be used immediately.
10. Do not use the lancet if the seal is broken as it may lead to infection at the site of skin puncture.
11. Follow the assay procedure strictly; any deviation will invalidate the results.
12. Do not reuse Test Device, lancets and plastic pipettes as it can lead to inaccurate results.
13. Do not touch the pipette tip to the surface of nitrocellulose membrane in the device.
14. Caution should be taken while interpreting results with potentially interfering samples like those from patients suffering from autoimmune disease such as Rheumatoid Arthritis.

PROCEDURE

ASSAY SETUP

Note: Ensure that the Test Device and Reaction Buffer are at *room temperature before starting the assay procedure.

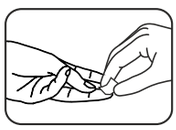
1. To open the Reaction Buffer dropper bottle, tighten the cap further to pierce the top of the nozzle of bottle and then remove the cap to deliver the drops of Reaction Buffer.
2. Remove the Test Device from pouch and label with the patient's identification number / name (as shown in figure).



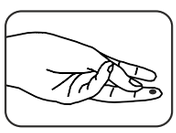
Note: Dispose off silica gel packets as mentioned in Precautions point no. 8.

ASSAY PROCEDURE

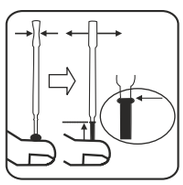
1. Select the finger for puncture, clean with sterile wipe and allow to air dry completely before puncturing the skin and collection of sample.



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



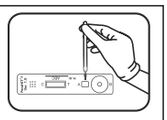
3. Aspirate blood sample with the help of disposable plastic pipette provided in the kit. Gently press the bulb of the pipette, immerse the open end into the blood drop and then gently release the pressure to aspirate blood upto the two projections (as shown in figure). Alternatively take 8 µL of sample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



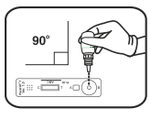
Note: Do not give excessive pressure on pipette bulb while aspirating blood.

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 round window 'B' of the Test Device.
   Note: It is important to hold reaction buffer in vertical position (90°) while adding reaction buffer in to buffer window.
   After adding each drop allow 10 seconds for soaking before adding the next drop.



5. Recap the Reaction Buffer bottle after adding Reaction Buffer to round window 'B' of the Test Device.
6. Read the test results between 20 to 30 minutes. Do not read beyond 30 minutes.
   READING TOO LATE CAN GIVE FALSE RESULTS.



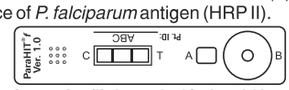
***Note : As per USP room temperature is (+15 °C to +30 °C).**

INTERPRETATION OF RESULTS

POSITIVE

Pf Positive

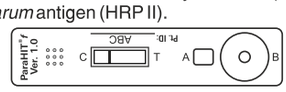
Appearance of two red coloured bands each at test (T) & control (C) region indicate the presence of *P. falciparum* antigen (HRP II).



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

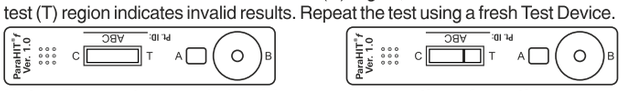
NEGATIVE

Appearance of single red coloured band only 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. The colour of control band should not be compared with colour of test band for interpretation of results.

DISPOSAL OF ASSAY DEVICE

Remnants of samples, used Test Device, pipettes, lancets, wipes and gloves should be discarded as biohazard waste as per local regulations.

LIMITATIONS OF THE TEST

1. The test procedure, precautions and interpretation of results for this test as given above 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 of blood 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. A positive test should be carefully interpreted to distinguish between a new infection and effectively treated old infection, which is due to the persistence of HRP II antigen in the blood for 1-5 weeks after effective treatment.[3]
6. Any modification to the above procedure and / or use of reagents other than provided in the kit would invalidate the test procedure.
7. In case of strong clinical evidence of malaria a negative test should be repeated.
8. This assay cannot be used for the diagnosis of infection by other types of malarial parasites viz. *P. malariae*, *P. vivax*, *P. ovale* or *P. knowlesi* which do not produce HRPII antigen.

QUALITY CONTROL

1. To ensure reliability of the test result it is recommended to follow kit's instructions meticulously.
2. Appearance of red coloured control line 'C' at the end of every test run validates the proper flow of reagents and performance of gold conjugate.
3. Every batch of **ParaHIT® f Ver. 1.0** - Device is released after confirming the sensitivity & specificity by running test with samples where the microscopy has confirmed the diagnosis of malaria.

PERFORMANCE CHARACTERISTICS[4]

The overall sensitivity and specificity of **ParaHIT® f Ver.1.0** - Immunochromatographic rapid, visual and an *in vitro* qualitative screening test for the detection of *Plasmodium falciparum* in whole blood to diagnose malaria was estimated by comparing its results with microscopy. Results of the evaluation study undertaken in various laboratories are summarised below.

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.

Laboratory	Sample size		% Sensitivity	% Specificity
	Malaria positive samples	Malaria negative samples		
Lab 1[4a]	173	981	99.42	99.59
Lab 2[4b]	78	50	100	92.7
Lab 3[4c]	83	192	85.5	98.9

B ANALYTICAL SENSITIVITY (Limit of detection)

The sensitivity of **ParaHIT® f Ver. 1.0** - Rapid Test for *P. falciparum* malaria is comparable to that of microscopic observation with 100 or more 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, P. vivax* and *P. knowlesi*.

D PRECISION

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

E RESIDUAL RISK : None

PRESENTATION

Reagent No.	Reagent Name	REF 55IC104-10 Σ 10	55IC104-25 25	55IC104-50 50	55IC104-25CH 25
Reagent 1	Test Device	10	25	50	25
Reagent 2	Reaction Buffer	2.5 mL	6 mL	12 mL	6 mL
Accessories (*QS - Quantity Sufficient)	Sterile Lancets	10	25	50	25
	Sterile Wipes	10	25	50	25
	Disposable Plastic Pipettes	10	25	50	25

REFERENCES

1. WHO Report, 2015
2. Howard R. J., et al. Secretion of a Malarial Histidine Rich Protein (PfHRP II) from *Plasmodium falciparum* - infected Erythrocytes. J.Cell. Biol., 1986; 103, 1269-1277.
3. WHO: Guidelines for Treatment of Malaria. Third Edition (2015) Annex 3 Malaria Diagnosis, 139.
4. Data on internal file.
   (a) Internal evaluation
   (b) External evaluation at National Health Laboratory Service, Johannesberg, South Africa; 2013
   (c) External evaluation at National Institute of Medical Research, Mwanza, Tanzania;2014

ACKNOWLEDGEMENT

We wishes to acknowledge that the technology for the manufacture of **ParaHIT® f Ver. 1.0** - Rapid Test for *P. falciparum* malaria was developed by the Program for Appropriate Technology in Health (PATH), Seattle, Washington, U.S.A. Funding for this research was provided by United States Agency for International Development (USAID), under Program for Advancement of Commercial Technology (PACT) for Child and Reproductive Health (CRH). The technology for the **ParaHIT® f Ver. 1.0** - Rapid Test for *P. falciparum* malaria has been licensed by PATH to us.

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
		CE	The product meets all the legal requirements for CE marking as per directive 98/79/EC

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.

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The Manufacturing site's QMS is Certified for
ISO 13485:2016, ISO 9001:2015

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ParaHIT® *f* ver. 1.0

Rapid Test for *P. falciparum* Malaria - Device

REF : 55IC116-10, 55IC116-25, 55IC116-50

Σ : 10, 25, 50

IVD +4 °C +40 °C

INTENDED USE

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Diagnosis of malaia depends on detection of parasites microscopically or detection of antigens derived from malarial parasites by immunological techniques. **ParaHIT® *f* Ver. 1.0 -** Rapid Test for *P. falciparum* malaria is designed 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[2]. 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) 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 antibody (detector antibody) binds to HRP II antigen which 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 antibody to HRP II (capture antibody) leading to formation of red coloured 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 (*QS - Quantity Sufficient)

Sterile Lancets (Twist Head Needle Type)

Sterile Wipes (70% Isopropyl alcohol impregnated)

Disposable Inverted Cup

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 until expiry in unopened condition whereas Reaction Buffer (Reagent 2) is stable until expiry in opened / unopened conditions. May be refrigerated. Do not freeze and protect the kit from direct sunlight. The shelf life of the kit is as indicated on the outer package.

BIOSAFETY

1. Handle all samples with care, as they can be potentially infectious.
2. Wear disposable gloves throughout the test procedure and dispose them off as biohazard waste.
3. Wear protective laboratory clothing in laboratory area.
4. Technicians with wound, cut or skin abrasions on the hand must refrain from performing the test without proper precautions like covering the wounds while working with samples.
5. Prevent splashing or spilling of samples or solutions containing samples. In case of spillage, immediately clean it with 1:10 dilution of freshly prepared 5% sodium hypochlorite solution and dispose off the cleaning material by a suitable method.
6. Any accessory which comes in direct contact with the specimen and used Test Devices should be considered as contaminated material and should be treated appropriately as per laboratory procedure.
7. Wash hands thoroughly with disinfectant after completion of the test.
8. After reading the results dispose off the Test Device & disposable Inverted cup as biohazard waste.

SPECIMEN COLLECTION AND HANDLING

Specimen	Storage at	Stability	Remarks
Whole blood (Capillary /Venous)	+2 °C to +8 °C	Short term (Upto 3 days)	If blood is refrigerated, allow it to come to room temperature prior to testing. Mix gently before testing. Using the specimen more than 3 days after collection can cause non-specific reaction. The test performs well with frozen sample if it is thawed only once.
	-20 °C or lower	Long term	

PRECAUTIONS

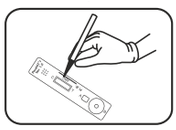
1. Do not use any specimen other than whole blood.
2. Do not use the reagents if they are unsealed or leaked or if the reagent bottle is not intact.
3. Do not use reagents after expiry date.
4. Do not combine the reagents from different lots of kits as they are optimized for individual lot to give best results.
5. Do not use the reagents, if they appear turbid or discoloured.
6. Open the sealed pouch at the time of assay performance only after it attains the room temperature.
7. Do not use the Test Device if the pouch is not intact.
8. On opening the pouch if the desiccant (silica gel) colour has changed to white, do not use that Device. Desiccant should be discarded as per local rules and regulations.
9. Once opened the Test Device should be used immediately.
10. Do not use the lancet if the seal is broken as it may lead to infection at the site of skin puncture.
11. Follow the assay procedure strictly; any deviation will invalidate the results.
12. Do not reuse Test Device, lancets and Disposable Inverted Cup as it can lead to inaccurate results.
13. Do not touch the Disposable Inverted Cup to the surface of nitrocellulose membrane in the device.
14. Caution should be taken while interpreting results with potentially interfering samples like those from patients suffering from autoimmune disease such as Rheumatoid Arthritis.

PROCEDURE

ASSAY SETUP

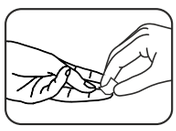
Note: Ensure that the Test Device and Reaction Buffer are at *room temperature before starting the assay procedure.

1. To open the Reaction Buffer dropper bottle, tighten the cap further to pierce the top of the nozzle of bottle and then remove the cap to deliver the drops of Reaction Buffer.
2. Remove the Test Device from pouch and label with the patient's identification number / name (as shown in figure).
   Note: Dispose off silica gel packets as mentioned in Precautions point no. 8.

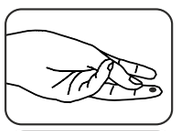


ASSAY PROCEDURE

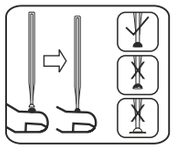
1. Select the finger for puncture, clean with sterile wipe and allow to air dry completely before puncturing the skin and collection of sample.



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



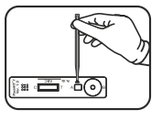
3. Transfer the blood sample with the help of disposable inverted cup. Immerse circular end of Inverted cup into the blood drop and transfer immediately to the square window 'A' of the device by touching the pad (as shown in the figure). Alternatively take 8 µl of sample with micro pipette and dispense immediately to the square window 'A' of the test device.



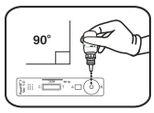
Note:

In case of venous whole blood, use micropipette for taking required quantity of blood and transferring to the Test Device.

4. Add 4 drops (200 µL) of Reaction Buffer to round window 'B' of the Test Device.
   Note: It is important to hold reaction buffer in vertical position (90°) while adding reaction buffer in to buffer window.
   After adding each drop allow 10 seconds for soaking before adding the next drop.



5. Recap the Reaction Buffer bottle after adding Reaction Buffer to round window 'B' of the Test Device.
6. Read the test results between 20 to 30 minutes. Do not read beyond 30 minutes.
   READING TOO LATE CAN GIVE FALSE RESULTS.



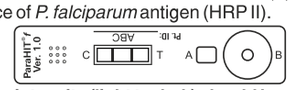
***Note : As per USP room temperature is (+15 °C to +30 °C).**

INTERPRETATION OF RESULTS

POSITIVE

Pf Positive

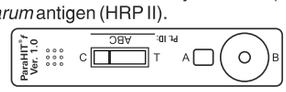
Appearance of two red coloured bands each at test (T) & control (C) region indicate the presence of *P. falciparum* antigen (HRP II).



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

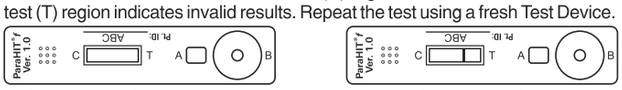
NEGATIVE

Appearance of single red coloured band only 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. The colour of control band should not be compared with colour of test band for interpretation of results.

DISPOSAL OF ASSAY DEVICE

Remnants of samples, used Test Device, Disposable Inverted Cup, lancets, wipes and gloves should be discarded as biohazard waste as per local regulations.

LIMITATIONS OF THE TEST

1. The test procedure, precautions and interpretation of results for this test as given above 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 of blood 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. A positive test should be carefully interpreted to distinguish between a new infection and effectively treated old infection, which is due to the persistence of HRP II antigen in the blood for 1-5 weeks after effective treatment.[3]
6. Any modification to the above procedure and / or use of reagents other than provided in the kit would invalidate the test procedure.
7. In case of strong clinical evidence of malaria a negative test should be repeated.
8. This assay cannot be used for the diagnosis of infection by other types of malarial parasites viz. *P. malariae*, *P. vivax*, *P. ovale* or *P. knowlesi* which do not produce HRPII antigen.

QUALITY CONTROL

1. To ensure reliability of the test result it is recommended to follow kit's instructions meticulously.
2. Appearance of red coloured control line 'C' at the end of every test run validates the proper flow of reagents and performance of gold conjugate.
3. Every batch of **ParaHIT® *f* Ver. 1.0** - Device is released after confirming the sensitivity & specificity by running test with samples where the microscopy has confirmed the diagnosis of malaria.

PERFORMANCE CHARACTERISTICS[4]

The overall sensitivity and specificity of **ParaHIT® *f* Ver.1.0** - Immunochromatographic rapid, visual and an *in vitro* qualitative screening test for the detection of *Plasmodium falciparum* in whole blood to diagnose malaria was estimated by comparing its results with microscopy. Results of the evaluation study undertaken in various laboratories are summarised below.

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.

Laboratory	Sample size		% Sensitivity	% Specificity
	Malaria positive samples	Malaria negative samples		
Lab 1[4a]	173	981	99.42	99.59
Lab 2[4b]	78	50	100	92.7
Lab 3[4c]	83	192	85.5	98.9

B ANALYTICAL SENSITIVITY (Limit of detection)

The sensitivity of **ParaHIT® *f* Ver. 1.0** - Rapid Test for *P. falciparum* malaria is comparable to that of microscopic observation with 100 or more 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, P. vivax* and *P. knowlesi*.

D PRECISION

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

E RESIDUAL RISK : None

PRESENTATION

Reagent No.	Reagent Name	REF 55IC116-10 / Σ 10	55IC116-25 25	55IC116-50 50
Reagent 1	Test Device	10	25	50
Reagent 2	Reaction Buffer	2.5 mL	6 mL	12 mL
Accessories (*QS - Quantity Sufficient)	Sterile Lancets	10	25	50
	Sterile Wipes	10	25	50
	Disposable Inverted Cup	10	25	50

REFERENCES

1. WHO Report, 2015
2. Howard R. J., et al. Secretion of a Malarial Histidine Rich Protein (PfHRP II) from *Plasmodium falciparum* - infected Erythrocytes. J.Cell. Biol., 1986; 103, 1269-1277.
3. WHO: Guidelines for Treatment of Malaria. Third Edition (2015) Annex 3 Malaria Diagnosis, 139.
4. Data on internal file.
   (a) Internal evaluation
   (b) External evaluation at National Health Laboratory Service, Johannesberg, South Africa; 2013
   (c) External evaluation at National Institute of Medical Research, Mwanza, Tanzania;2014

ACKNOWLEDGEMENT

We wishes to acknowledge that the technology for the manufacture of **ParaHIT® *f* Ver. 1.0** - Rapid Test for *P. falciparum* malaria was developed by the Program for Appropriate Technology in Health (PATH), Seattle, Washington, U.S.A. Funding for this research was provided by United States Agency for International Development (USAID), under Program for Advancement of Commercial Technology (PACT) for Child and Reproductive Health (CRH). The technology for the **ParaHIT® *f* Ver. 1.0** - Rapid Test for *P. falciparum* malaria has been licensed by PATH to us.

SYMBOL LEGENDS

Symbol	Explanation of Symbol
	Consult instructions for use
	Do not use if package is damaged
IVD	*In vitro* diagnostic device
+4 °C +40 °C	Store at +4 °C to +40 °C
	Keep away from sunlight
	Keep dry
	Do not reuse

Symbol	Explanation of Symbol
Σ	Contains sufficient for 'n' tests
REF	Catalogue number
LOT	Batch code No.
	Manufacturer
	Date of manufacture
	Use by (date or month of expiry)

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Web: www.arkray.co.in

The Manufacturing site's QMS is Certified for
ISO 13485:2016, ISO 9001:2015

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