WHO's performance evaluation of in vitro diagnostics
Performance evaluation public assessment report (PEPAR)

Product: Lariacheck Pf- Rapid Test for *P.falciparum* Malaria Device
Orchid Biomedical Systems – A Division of Tulip Diagnostics (P) Ltd

Lariacheck Pf- Rapid Test for *P.falciparum* Malaria Device with product codes 302100025(1T), 302100005, 302100010, 302100025 and 302100100, manufactured by Orchid Biomedical Systems – A Division of Tulip Diagnostics (P) Ltd, was found by the World Health Organization (WHO) to meet the technical requirements for WHO's performance evaluation of in vitro diagnostics (IVDs) on 1 May 2023[1]. This outcome does not mean or imply that such IVD will be accepted for WHO's prequalification assessment pursuant to the separate procedure applicable thereto and/or be granted WHO's prequalification listing.

Summary of WHO's performance evaluation

Within the framework of WHO's performance evaluation procedure for IVDs[2], Lariacheck Pf- Rapid Test for *P.falciparum* Malaria Device was evaluated by the Centers for Disease Control and Prevention in the fourth quarter of 2022, according to protocol PQDx_317, version 3.1. The evaluation was conducted using product code 302090025 and instructions for use (IFU) version 1022/VER-03, date of issue 2021-08, as included in Annex 2. This was a previous version of the IFU compared to the IFU version submitted in this expression of interest (EOI) and it referred to product name "Paracheck Pf Rapid Test for *P. falciparum* Malaria (Ver.5)", which is the previous name of the product. The test procedure and interpretation is identical in both IFU versions. The IFU submitted in this EOI for product codes 302100025(1T), 302100005, 302100010, 302100025 and 302100100 is included in Annex 1.

Lariacheck Pf- Rapid Test for *P.falciparum* Malaria Device was evaluated against a *Plasmodium falciparum* cultured line panel, *P. falciparum* wild type parasite panel, *P. vivax* wild type parasite panel, and a *P. falciparum*-, *P.vivax*-negative panel (including clean negatives from afebrile donors and specimens with possible cross-reactants), and the WHO International Standard for *P. falciparum* antigens.

[1] This evaluation was carried out prior to the implementation of WHO's performance evaluation procedure in 2026. However, based on the declaration from the manufacturer that the product in the previous submission is identical, except for IFU version, to the product submitted in the EOI, these results were considered for this EOI.

[2] Refer to document PQDx_458 "*WHO's performance evaluation procedure for in vitro diagnostics*"

Performance characteristics			
	P. falciparum	***P. vivax***	**Pf *-hrp2* deletion panel**
Panel detection score[a] at 200 parasites/µL (N=100)	86/100, 86.0%	*NA for Pf assays*	*NA*
Proportion reactive lines at 200 parasites/µL	HRP2 line: 90.0% (N=400)	*NA*	*NA*
False positive results	All negative specimens: 0/200, 0.0% Of which, clean negative specimens: 0/104, 0 % No false Pf result on the *P. vivax* specimens at 200 and 2000 parasites/µL		
Invalid rate % (N= 1082)	0/1082, 0 %		
Inter-reader variability on the wild type and negative panels % (N= 1010)	30/1010, 3 %		
Lowest concentration of HRP2/pLDH detected using the 1st WHO International standard for Pf antigens (NIBSC code: 16/376)*	Lot A: 15.6 IU/mL Lot B: 15.6 IU/mL		

[a] *The panel detection score is the proportion of specimens at a specified concentration that gave positive results in all 4 tests performed with this specimen (2 tests on each of 2 lots)*

Operational characteristics and ease of use

This assay does not require laboratory equipment and can be performed in laboratories with limited facilities or in non-laboratory settings.

The assay was found easy to use by the operators performing the evaluation.

However, the operators noted that 212/1010 (21.0 %) of the tests performed on the wild-type and negative panels showed anomalies (207 with incomplete clearing and 4 with red background, and 1 with red background obscuring test line).

Key operational characteristics	
Specimen types and volume	5 µL of capillary or venous whole blood (EDTA, Heparin or Oxalate anticoagulants)
Number of steps*	2 steps in total 1 step with specimen transfer device (precision pipette was used during the evaluation)
Time to result	20 minutes
Endpoint stability (interval)	10 minutes (the test can be read between 20 and 30 minutes after addition of diluent)

Internal QC	Yes, reagent addition control

** Definition: each action required to obtain a result (excluding specimen collection, device preparation – opening the pouch), e.g. for RDTs: add specimen, add buffer (2 steps).*

Based on these results, WHO has found that Lariacheck Pf- Rapid Test for *P.falciparum* Malaria Device meets the technical requirements for WHO's performance evaluation of IVDs.

Annex 1 Instructions for use

Products submitted in this Expression of Interest

(product codes 302100025(1T), 302100005, 302100010, 302100025 and 302100100)

Lariacheck™ Pf

RAPID TEST FOR *P. FALCIPARUM* MALARIA

DEVICE

INTENDED USE

Lariacheck Pf™ is an *invitro*, rapid, qualitative, two site sandwich immunoassay for the determination of *P. falciparum* specific histidine rich protein - 2 (Pf. HRP-2) in whole blood (capillary or venous) for the diagnosis of falciparum malaria in individuals with signs and symptoms consistent with malaria infection. The test is intended for healthcare professionals at the clinical setup and point of care sites.

SUMMARY

Four species of the Plasmodium parasites are responsible for malaria infections in humans viz. *P. falciparum, P. vivax, P. ovale* and *P. malariae*. Of these *P. falciparum* is the most prevalent and severe species that is responsible for most of the morbidity and mortality worldwide. Early detection of *P. falciparum* malaria is of paramount importance due to incidence of cerebral malaria and drug resistance associated with it. Pf. HRP-2 is a water soluble protein that is released from parasitised erythrocytes of infected individuals and is specific to the *P. falciparum* species.
Lariacheck Pf™ detects the presence of Pf. HRP-2 in venous or capillary whole blood specimen and is a sensitive and specific test for the detection of *P. falciparum* malaria.

PRINCIPLE

Lariacheck Pf™ utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. As the test specimen flows through the membrane assembly of the device after addition of the clearing buffer, the colored Agglutinating Sera for HRP-2-colloidal gold conjugate complexes the HRP-2 in the lysed specimen. This complex moves further on the membrane to the test region where it is immobilised by the Agglutinating Sera for HRP-2 coated on the membrane leading to formation of a colored band which confirms a positive test result. Absence of this colored band in the test region indicates a negative test result. The unreacted conjugate and unbound complex if any, move further on the membrane and are subsequently immobilised by the Agglutinating Sera for Rabbit globulin coated on the membrane at the control region, forming a colored band. The control band formation is based on the 'Rabbit globulin / Agglutinating Sera for Rabbit globulin' system. Since it is completely independent of the analyte detection system, it facilitates formation of consistent control band signal independent of the analyte concentration. This control band serves to validate the test performance.

REAGENTS AND MATERIALS SUPPLIED

Lariacheck Pf™ kit contains :

A. Individual pouches, each containing :
   1. DEVICE Membrane assembly predispensed with Agglutinating Sera for HRP-2 - colloidal gold conjugate, rabbit globulin-colloidal gold conjugate, Agglutinating Sera for HRP-2 and Agglutinating Sera for rabbit globulin at the respective regions.
   2. Desiccant pouch.
B. INVERTED CUP Disposable 5µl specimen applicator.
C. BUF Clearing buffer containing surfactant and preservative in a dropper bottle.
D. Instructions for Use.
E. Pictorial representation.
F. Alcohol swabs.
G. Sterile lancets.

Product codes REF	302100025(1T)	302100005	302100010	302100025	302100100
Pouch sealed tests	25 x 1T	05	10	25	100
Disposable specimen applicators	25	05	10	25	100
Clearing buffer bottles	25 x 1.25ml	01 x 1.5ml	01 x 3.0ml	01 x 4.0ml	04 x 4.0ml
Alcohol swabs	25	05	10	25	100
Sterile lancets	25	05	10	25	100
Pictorial Instruction for use	25	01	01	01	01
Instruction for use	25	01	01	01	01

MATERIALS REQUIRED BUT NOT PROVIDED

Calibrated micropipette capable of delivering 5µl specimen accurately, disposable micropipette tips. Permanent marker Pen/pencil, disposable gloves, timer. Biosafety sharps container and Biohazard waste container (for potentially infectious waste). Venipuncture blood collection kit (if whole blood is collected by venepuncture). Additional alcohol swabs (if any included in the kit are found dry) and additional sterile lancets (if any included in the kit misfire/ do not produce sufficient blood volume).

STORAGE AND STABILITY

The test kit may be stored between 4°C To 45°C till the duration of the shelf life as indicated on the pouch / carton.
After first opening of the clearing buffer, the buffer is stable until the expiry date mentioned on the vial label, if kept at 4°C To 45°C. DO NOT FREEZE the kit or components.

WARNINGS AND PRECAUTIONS

Read the instructions carefully before performing the test.
For *in vitro* diagnostic use only. NOT FOR MEDICINAL USE. For professional use.
The test is not intended for use in screening of asymptomatic individuals or for monitoring of success of therapy.
Do not use beyond expiry date.
Do not intermix components of one kit with another.
Handle all specimens as potentially infectious.
Follow standard biosafety guidelines for handling and disposal of potentially infective material.
Clearing buffer contains Sodium Azide (0.095%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build up in the plumbing.
The test device is intended for SINGLE USE ONLY.
Reduced light conditions increase risk of errors during testing and interpretation of test results. Make sure that the test performance and test interpretation is carried out in sufficient light conditions.
Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.

SPECIMEN COLLECTION AND PREPARATION

Fresh anti coagulated whole blood should be used as test specimen and EDTA or Heparin or Citrate or CPDA or Oxalate can be used as suitable anticoagulant. The specimen should be collected in a clean glass or plastic container. If immediate testing is not possible then specimen may be stored at 2°C To 8°C for upto 72 hours before testing. For long-term storage, freeze the specimen below -20°C. Repeated freezing and thawing of the specimen should be avoided (Maximum of 2 freeze/thaw cycles are allowed). Thawed specimens must be mixed gently prior to testing. Hemolysed, clotted or contaminated or blood specimen with bubbles should not be used for performing the test. Fresh blood from finger prick / puncture may also be used as a test specimen.

TEST PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the **Lariacheck Pf**™ kit components to room temperature before testing.
2. Check the expiration date of the kit (including buffer). If expired, do not use but take another unexpired kit.

3. Check that the cassette packaging is not damaged. If damaged, discard the test and use another test.
4. In case the pouch has been stored at 2°C To 8° C allow atleast 30 minutes for the device to come to room temperature.
5. Open the pouch and retrieve the device and desiccant. Check the colour of the desiccant. It should be blue. If it has turned colourless or pink discard the device and use another device. ***Once opened, the device must be used immediately.***
6. Label the device with patient name or identifier.
7. Put on gloves. Use new gloves for each patient.
8. For Capillary whole blood from finger prick
    1. Wear gloves.
    2. Choose a finger for the finger prick: Do not choose a finger that is swollen, bruised or scarred. Preferably choose the 3rd or 4th finger of the hand the patient does not use to write.
    3. Open the packaging of the alcohol swab. Take out the alcohol swab. Do not throw away the empty packaging (wrapper) but keep it aside.
    4. Wipe the complete fingertip with the alcohol swab. Wait until the finger has completely dried (minimum 30 seconds).
    5. Place the alcohol swab in the wrapper and set it aside (you will use it again to stop the bleeding after you collected the patient's blood).
    6. Take the sterile lancet.
    7. Twist and remove the rounded cap of the sterile lancet, taking care not to touch the tip/ point. Puncture the side of the pulp (ball) of the finger with the lancet, perpendicular to the lines of the fingerprint. Dispose the lancet immediately into the sharps box.
    8. Make sure a well-formed drop of blood is present.
    9. If there is no well-formed drop of blood, repeat the finger prick using a new lancet and choose a different puncture site.
    10. Take the specimen applicator and collect 5 µl of blood in the circular end of the specimen applicator into the whole blood drop.
    11. Place the circular end of the specimen applicator in the rectangular well (marked "A") so that it touches the strip (pad at the bottom of the well).Press down lightly to transfer all the blood to the strip. Put the used specimen applicator into the non-sharps disposal container for potentially infectious waste.
    12. Take the alcohol swab you put aside (step 5).Ask the patient to press it to the finger prick to stop the bleeding. After use, put the alcohol swab into the non-sharps disposal container for potentially infectious waste.
    13. Take the buffer bottle. Hold the open buffer bottle vertically above the circular well (marked "B").
    In a vertical position, squeeze the buffer bottle gently and apply exactly 2 drops into the circular well (marked "B") without contacting the device to avoid contamination.
    14. Remove your gloves and discard them into the non-sharps disposal container for potentially infectious waste.
    15. Write the time on the cassette or set a countdown timer to the required reading time.
    16. Read test results after a minimum of 20 minutes but no later than 30 minutes. Use a good light source when reading the test results.

For Venous whole blood from venipuncture

1. Wear gloves.
2. Collect blood by standard venipuncture procedure into a tube containing the correct anticoagulant (EDTA, heparin, citrate, CPDA or oxalate).
3. Mix the tube gently.
4. Transfer 5 µl of whole blood in the rectangular well (marked "A") of the cassette using a precision pipette.
5. Perform steps 12–16 of the previous section ("Capillary whole blood from finger prick").

INTERPRETATION OF RESULTS

NEGATIVE for *P. falciparum* malaria: A colored band appears in the control window 'C'.

POSITIVE for *P. falciparum* malaria : In addition to the control band, a distinct colored band also appears in the test window 'T'. Appearance of coloured band of any intensity (faint to dark) at 'T' should be considered as positive result for *P. falciparum* malaria.

INVALID : The test should be considered invalid if no colored band appears on the device. The test should also be considered invalid if a colored band appears only at the test window 'T' and not at the control window 'C'. In such cases, repeat the test with a new device, ensuring that the test procedure has been followed accurately.

QUALITY CONTROL RECOMMENDATIONS

To control proper test performance, it is recommended to include internal positive and negative control specimens.

PERFORMANCE CHARACTERISTICS

A. Analytical Performance Study

A1 . Potentially interfering exogenous and endogenous substances:

The following Potentially interfering substances have no impact on the test results of **Lariacheck Pf**™.

Type of Specimen	Sr. No	Potential Interfering substance
Endogenous Substances	1	Total Protein
	2	Bilirubin, conjugated
	3	Cholesterol
	4	Triglycerides
	5	Haemoglobin
Anti- Malaria Drugs	1	Chloroquine
	2	Doxycycline
	3	Sulfadoxine
	4	Pyrimethamine
	5	Mefloquine
Antibiotics	1	Amoxycilin
	2	Ciprofloxacin
Anti - inflammatory Drugs	1	Aspirin
	2	Ibuprofen
Anti- TB Drugs	1	Ethambutol
	2	Isoniazid
	3	Rifampin
Anti -Retroviral Drugs	1	Lamivudine
	2	Efavirenz
	3	Emtricitabine
	4	Tenofovir
	5	Atazanavir
Exogenous substances	1	Ethanol
	2	Caffeine

A2. Cross Reacting infections, disease and medical conditions:
The following 20 Potentially cross reacting infections/disease/conditions did not affect the performance of **Lariacheck Pf™**.

Potentially cross reacting infections/disease/conditions			
1	Chagas AB Positive Plasma	11	Leptospira antibody positive sera
2	Dengue positive sera for IgG & IgM Antibodies	12	Syphillis positive plasma
3	Measles Positive Plasma	13	Leishmania spp
4	HAV antibody positive plasma	14	Brucella spp
5	HBV positive plasma	15	Tick borne encephalitis
6	HCV antibody positive plasma	16	Schistosoma sp.
7	HIV antibody positive plasma	17	HAMA
8	Toxoplasma antibody positive plasma	18	ANA
9	Influenza A IgM / A IgG / B antibody positive plasma	19	Rheumatoid factor
10	Yellow fever vaccinated sera	20	Recipients of multiple blood transfusion and Pregnant women (including multiparous)

A3.Precision(Repeatability)
Within run, precision was determined using 10 replicates of 5 different venous whole blood specimens in 03 different lots of **Lariacheck Pf™** which is summarized below:

*Quality control Panel	Accuracy(%)
Malaria Negative	100%
P.falciparum Positive (Moderate Positive)	100%
P.falciparum Positive (Weak Positive)	100%
P.vivax Positive (Moderate Positive)	100%
P.vivax Positive (Weak Positive)	100%

A4.Precision (Reproducibility)
Between run, precision was determined using 5 different blinded venous whole blood specimen in 03 different lots of **Lariacheck Pf™** by 3 different operators at 3 different sites on 5 different days which is summarized below:

*Quality control Panel	Accuracy(%)			
	Between Day	Between Operators	Between Lot	Between Site
Malaria Negative	100%	100%	100%	100%
P.falciparum Positive (Moderate Positive)	100%	100%	100%	100%
P.falciparum Positive (Weak Positive)	100%	100%	100%	100%
P.vivax Positive (Moderate Positive)	100%	100%	100%	100%
P.vivax Positive (Weak Positive)	100%	100%	100%	100%

*Quality control panel specimens have been confirmed by microscopy as malaria negative and malaria positive. Malaria positive specimens were classified as moderate or weak positive based on respective parasite counts as determined by microscopy.
There was no variation in test results at different sites under uncontrolled condition of temperature and RH upto 88%.

A5. Analytical Sensitivity
Lariacheck Pf™ Device detects NIBSC standard for *Plasmodium falciparum* antigens (HRP2) NIBSC Code :16/376 upto concentration of 7.8 IU/ml.

A6. High dose hook effect
The product **Lariacheck Pf™** Device-Rapid Test for *P.falciparum* malaria does not show High dose hook effect up to parasite counts of 378600 for *P.falciparum*.

B. Clinical Performance study :

B1. External evaluation studies:

Study 1

Table 1

Study Site	Total Number of specimens tested	Specimen Type		Number of specimens negative by Microscopy	Number of specimens negative in Lariacheck Pf™	Number of specimens specimens falsely positive in Lariacheck Pf™	Specificity (95%CI)
		Population type	Mode of collection				
Jharkhand, India	7428	Patient with fever	Finger prick/Venous Phlebotomy	7428	7428	0	100% (99.95% to 100%)

Table 2

Study Site	Total Number of specimens tested	Population type	Specimen Type		Number of specimens positive by Microscopy	Number of specimens positive in Lariacheck Pf™	Number of specimens falsely positive in Lariacheck Pf™	Sensitivity (95%CI)
			Mode of collection	Species type				
Jharkhand, India	421	Patient with fever	Finger prick/ Venous Phlebotomy	*P. falciparum*	421	421	0	100% (99.13 to100%)
	437			*P. vivax*	437	0	0	-
	14			*P. falciparum + P. vivax*	14	14	0	100% (76.84% to 100%)

Study 2

Sr.No.	Test Country	Total Test	True Pos	False Pos	False Neg	Neg	Sensitivity	Specificity	Accuracy
01	Msambweni Country Referral Hospital Kenya	207	43	0	0	164	100% (91.78% to 100.00%)	100% (97.78% to 100.00%)	100% (98.23% to 100.00%)
02	Sio port sub-country Hospital Kenya	259	56	0	0	203	100% (93.62% to 100.00%)	100% (98.20% to 100.00%)	100% (98.59% to 100.00%)

LIMITATIONS OF THE TEST

1. As with all diagnostic tests, the test result must always be correlated with clinical findings. Negative results must be confirmed by microscopic examination of thick smear and thin blood films. As is often done in serial microscopy testing, another specimen may be collected and tested.
2. A positive result must be verified with a confirmation test.
3. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
4. Interference due to presence of heterophile antibodies in patient's specimen can lead to erroneous analyte detection in immunoassay, has been reported in various studies. **Lariacheck Pf**™ uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of this interference.
5. In *P. falciparum* malaria infection, HRP-2 is not secreted in gametogony stage. Hence, in "Carriers", the HRP-2 band may be absent.
6. Since the Pf. HRP-2 persists for upto a fortnight even after successful therapy, a positive test result does not indicate a failed therapeutic response.
7. Do not interpret the test results beyond 30 minutes.
8. Use of old blood specimens may lead to reddish or unclear background on the test device.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1) Howard, R. J., et al., 1986 : Secretion of Malarial Histidine-Rich Protein (Pf. HRP II) from *Plasmodium falciparum*-infected Erythrocytes. J. Cell Biol., 103, 1269-1277. (2) Rock, E.P., et al., 1987 : Comparative Analysis of the *Plasmodium falciparum* Histidine-Rich Proteins HRP-I, HRP-II and HRP-III in Malaria Parasites of Diverse Origin. Parasitol., 95, 209-227. (3) Parra, M.E., et al., 1991 : Identification of *Plasmodium falciparum* Histidine-Rich Protein 2 in the Plasma of Humans with Malaria, J. Clin. Microbiol., 29, 1629-1634. (4) Rodriguez-Del Valle, M., et al., 1991 : Detection of Antigens and Antibodies in the Urine of Humans with *Plasmodium falciparum* Malaria. J. Clin. Microbiol., 29, 1236-1242. (5) Data on file : Orchid Biomedical Systems.

SYMBOL KEYS

	Temperature Limitation		Manufacturer	DEVICE	Device		This side up
	Use by		Consult Instructions for use	Σ	Contains sufficient for <n> tests		Do not reuse
	Date of Manufacture	REF	Catalogue Number	BUF	Clearing Buffer		
LOT	Batch Number / Lot Number	IVD	In vitro Diagnostic Medical Device	INVERTED CUP	Disposable Plastic Specimen Applicator		

Manufactured by:
Orchid Biomedical Systems
A Division of Tulip Diagnostics (P) Ltd.
88/89, Phase II C, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.
Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.
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PI/30210/0326/VER-DRAFT
Date of issue:

Annex 2 Instructions for use

Provided in kits for the performance evaluation

CE

paracheck® Pf

RAPID TEST FOR *P. FALCIPARUM* MALARIA (Ver.5)

DEVICE

INTENDED USE

paracheck Pf® is an *invitro*, rapid, qualitative, two site sandwich immunoassay for the determination of *P. falciparum* specific histidine rich protein - 2 (Pf. HRP-2) in whole blood (capillary or venous) for the diagnosis of falciparum malaria in individuals with signs and symptoms consistent with malaria infection. The test is intended for healthcare professionals at the clinical setup and point of care sites.

SUMMARY

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paracheck Pf® kit contains :

A. Individual pouches, each containing :
   1. DEVICE Membrane assembly predispensed with Agglutinating Sera for HRP-2 - colloidal gold conjugate, rabbit globulin-colloidal gold conjugate, Agglutinating Sera for HRP-2 and Agglutinating Sera for rabbit globulin at the respective regions.
   2. Desiccant pouch.
B. PIPETTE Disposable 5µl specimen applicator.
C. BUF Clearing buffer containing surfactant and preservative in a dropper bottle.
D. Instructions for Use.
E. Pictorial representation.
F. Alcohol swabs.
G. Sterile lancets.

REF	302090001	302090005	302090010	302090025	302090100
Σ	1	5	10	25	100

MATERIALS REQUIRED BUT NOT PROVIDED

Calibrated micropipette capable of delivering 5µl specimen accurately, disposable micropipette tips. Permanent marker Pen/pencil, disposable gloves, timer. Biosafety sharps container and Biohazard waste container (for potentially infectious waste). Venipuncture blood collection kit (if whole blood is collected by venepuncture). Additional alcohol swabs (if any included in the kit are found dry) and additional sterile lancets (if any included in the kit misfire/ do not produce sufficient blood volume).

STORAGE AND STABILITY

The test kit may be stored between 4°C To 45°C till the duration of the shelf life as indicated on the pouch / carton.
After first opening of the clearing buffer, the buffer is stable until the expiry date mentioned on the vial label, if kept at 4°C To 45°C. DO NOT FREEZE the kit or components.

WARNINGS AND PRECAUTIONS

Read the instructions carefully before performing the test.
For *in vitro* diagnostic use only. NOT FOR MEDICINAL USE. For professional use.
The test is not intended for use in screening of asymptomatic individuals or for monitoring of success of therapy.
Do not use beyond expiry date.
Do not intermix components of one kit with another.
Handle all specimens as potentially infectious.
Follow standard biosafety guidelines for handling and disposal of potentially infective material.
Clearing buffer contains Sodium Azide (0.095%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build up in the plumbing.
The test device is intended for SINGLE USE ONLY.
Reduced light conditions increase risk of errors during testing and interpretation of test results. Make sure that the test performance and test interpretation is carried out in sufficient light conditions.
Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.

SPECIMEN COLLECTION AND PREPARATION

Fresh anti coagulated whole blood should be used as test specimen and EDTA or Heparin or Oxalate can be used as suitable anticoagulant. The specimen should be collected in a clean glass or plastic container. If immediate testing is not possible then specimen may be stored at 2° C To 8°C for upto 72 hours before testing. For long-term storage, freeze the specimen below -20ºC. Repeated freezing and thawing of the specimen should be avoided (Maximum of 2 freeze/thaw cycles are allowed). Thawed specimens must be mixed gently prior to testing. Hemolysed, clotted or contaminated blood specimens should not be used for performing the test. Fresh blood from finger prick / puncture may also be used as a test specimen.

TEST PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the **paracheck Pf**® kit components to room temperature before testing.
2. Check the expiration date of the kit (including buffer). If expired, do not use but take another unexpired kit.
3. Check that the cassette packaging is not damaged. If damaged, discard the test and use another test.
4. In case the pouch has been stored at 2°C To 8° C allow atleast 30 minutes for the device to come to room temperature.
5. Open the pouch and retrieve the device, specimen applicator and desiccant. Check the colour of the desiccant. It should be blue. If it has turned colourless or pink discard the device and use another device. ***Once opened, the device must be used immediately.***
6. Label the device with patient name or identifier.
7. Put on gloves. Use new gloves for each patient.
8. For Capillary whole blood from finger prick
    1. Wear gloves.
    2. Choose a finger for the finger prick: Do not choose a finger that is swollen, bruised or scarred. Preferably choose the 3rd or 4th finger of the hand the patient does not use to write.
    3. Open the packaging of the alcohol swab. Take out the alcohol swab. Do not throw away the empty packaging (wrapper) but keep it aside.
    4. Wipe the complete fingertip with the alcohol swab. Wait until the finger has completely dried (minimum 30 seconds).
    5. Place the alcohol swab in the wrapper and set it aside (you will use it again to stop the bleeding after you collected the patient's blood).
    6. Take the sterile lancet.
    7. Detach the sterile lancet from the pouch, taking care not to touch the tip/ point. Puncture the side of the pulp (ball) of the finger with the lancet, perpendicular to the lines of the fingerprint. Dispose the lancet immediately into the sharps box.
    8. Make sure a well-formed drop of blood is present.
    9. If there is no well-formed drop of blood, repeat the finger prick using a new lancet and choose a different puncture site.
    10. Take the specimen applicator and collect 5 µl of blood by dipping the circular end of the specimen applicator into the whole blood drop.
    11. Place the circular end of the specimen applicator in the rectangular well (marked "A") so that it touches the strip (pad at the bottom of the well).Press down lightly to transfer all the blood to the strip. Put the used specimen applicator into the non-sharps disposal container for potentially infectious waste.
    12. Take the alcohol swab you put aside (step 5).Ask the patient to press it to the finger prick to stop the bleeding. After use, put the alcohol swab into the non-sharps disposal container for potentially infectious waste.
    13. Take the buffer bottle. Hold the open buffer bottle vertically above the circular well (marked "B"). In a vertical position, squeeze the buffer bottle gently and apply exactly 2 drops into the circular well (marked "B") without contacting the device to avoid contamination.
    14. Remove your gloves and discard them into the non-sharps disposal container for potentially infectious waste.

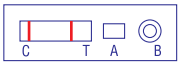
15. Write the time on the cassette or set a countdown timer to the required reading time.
16. Read test results after a minimum of 20 minutes but no later than 30 minutes. Use a good light source when reading the test results.

For Venous whole blood from venipuncture

1. Wear gloves.
2. Collect blood by standard venipuncture procedure into a tube containing the correct anticoagulant (EDTA, heparin or oxalate).
3. Mix the tube gently.
4. Transfer 5 µl of whole blood in the rectangular well (marked "A") of the cassette using a precision pipette.
5. Perform steps 12–16 of the previous section ("Capillary whole blood from finger prick").

INTERPRETATION OF RESULTS

NEGATIVE for *P. falciparum* malaria: A colored band appears in the control window 'C'.



POSITIVE for *P. falciparum* malaria : In addition to the control band, a distinct colored band also appears in the test window 'T'.

INVALID : The test should be considered invalid if no colored band appears on the device. The test should also be considered invalid if a colored band appears only at the test window 'T' and not at the control window 'C'. In such cases, repeat the test with a new device, ensuring that the test procedure has been followed accurately.

QUALITY CONTROL RECOMMENDATIONS

To control proper test performance, it is recommended to include internal positive and negative control specimens.

PERFORMANCE CHARACTERISTICS

A. Clinical Performance study : Diagnostics Specificity and Diagnostics Sensitivity

A1. In an in-house study ,a panel of 150 venous whole blood specimens whose results were earlier confirmed with microscopy were tested with **paracheck Pf®**. The results obtained are as follows:

Specimens	Total no. of specimens tested	paracheck Pf®		Sensitivity (95%CI)	Specificity (95%CI)
		Positive	Negative		
P.falciparum	25	25	0	100% (86.28% to 100%)	-
P. vivax	25	25	0	100% (86.28% to 100%)	-
Malaria Negative	100	0	100	-	100% (96.38% to 100%)

A2. External evaluation studies:

Table 1

Study Site	Total Number of specimens tested	Specimen Type		Number of specimens negative by Microscopy	Number of specimens negative in paracheck Pf®	Number of specimens falsely positive in paracheck Pf®	Specificity (95%CI)
		Population type	Mode of collection				
Jharkhand, India	7428	Patient with fever	Finger prick/ Venous Phlebotomy	7428	7428	0	100% (99.95% to 100%)

Table 2

Study Site	Total Number of specimens tested	Population type	Specimen Type		Number of specimens positive by Microscopy	Number of specimens positive in paracheck Pf®	Number of specimens falsely positive in paracheck Pf®	Sensitivity (95%CI)
			Mode of collection	Species type				
Jharkhand, India	421	Patient with fever	Finger prick/ Venous Phlebotomy	*P.falciparum*	421	421	0	100% (99.13 to100%)
	437			*P.vivax*	437	437	0	100% (99.16% to 100%)
	14			*P.falciparum* + *P.vivax*	14	14	0	100% (76.84% to 100%)

LIMITATIONS OF THE TEST

1. As with all diagnostic tests, the test result must always be correlated with clinical findings. Negative results must be confirmed by microscopic examination of thick smear and thin blood films. As is often done in serial microscopy testing, another specimen may be collected and tested.
2. A positive result must be verified with a confirmation test.
3. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
4. Interference due to presence of heterophile antibodies in patient's specimen can lead to erroneous analyte detection in immunoassay, has been reported in various studies. **paracheck Pf®** uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of this interference.
5. In *P. falciparum* malaria infection, HRP-2 is not secreted in gametogony stage. Hence, in "Carriers", the HRP-2 band may be absent.
6. Since the Pf. HRP-2 persists for upto a fortnight even after successful therapy, a positive test result does not indicate a failed therapeutic response.
7. Do not interpret the test results beyond 30 minutes.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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SYMBOL KEYS

Temperature Limitation	Manufacturer	DEVICE — Device	Contains sufficient for <n> tests
Use by	Consult Instructions for use	PIPETTE — Disposable Plastic Specimen Applicator	Do not reuse
Date of Manufacture	REF — Catalogue Number	BUF — Clearing Buffer	This side up
LOT — Batch Number / Lot Number	IVD — In vitro Diagnostic Medical Device	EC REP — Authorised Representative in the European Community	

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EC REP
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