

DUOCHECK Pf™

Rapid Test For Malaria HRP-2 (P.f) and pLDH (P.f) Antigen

DEVICE

INTENDED USE

DUOCHECK Pf™ is an invitro, rapid, qualitative, two site sandwich immunoassay for the combined determination of *P. falciparum* specific histidine rich protein - 2 (P.f HRP-2) and *P. falciparum* specific pLDH (P.f pLDH) 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

Five species of the Plasmodium parasites are responsible for malaria infections in human viz. *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Early detection of malaria is of utmost importance due to incidence of cerebral malaria and drug resistance associated with *falciparum* malaria and due to the morbidity associated with the other malarial forms.

It has been observed that emergence of *P. falciparum* parasites with deletions in the P.f HRP-2 and P.f HRP-3 genes in most malaria-endemic regions poses a significant biological threat to malaria diagnosis. These parasites are undetectable by HRP-2-based rapid diagnostic tests, – resulting in false-negative results, which can have lethal consequences for the management of malaria patients. To overcome such biological challenges, it is recommended to add P.f pLDH detection along with P.f HRP-2 for diagnosis of *P. falciparum* malaria.

DUOCHECK Pf™ detects *P. falciparum* malaria, utilises the specific differential detection of *P. falciparum* specific histidine rich protein-2 (P.f HRP-2) and *P. falciparum* specific Plasmodium lactate dehydrogenase (P.f pLDH) in venous or capillary whole blood specimen and is a sensitive and specific test for the detection of *P. falciparum* malaria.

PRINCIPLE

DUOCHECK Pf™ test 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 P.f HRP-2-colloidal gold and P.f pLDH conjugate complexes the HRP-2 and P.f pLDH 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 and Pan pLDH coated on the membrane leading to formation of a colored band which confirms a positive test result in respective test band areas marked as 'H' and 'L'. 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

DUOCHECK Pf™ kit contains:

- Individual pouches, each containing:
 - DEVICE** Membrane assembly pre-dispensed with Agglutinating Sera for HRP-2 colloidal gold conjugate, P.f pLDH colloidal gold conjugate, rabbit globulin-colloidal gold conjugate, Agglutinating Sera for HRP-2 and pLDH and Agglutinating Sera for rabbit globulin at the respective regions.
 - Desiccant pouch.
- INVERTED CUP** Disposable 5µl specimen applicator.
- BUF** Clearing buffer containing surfactant and preservative in a dropper bottle.
- Instructions for Use.
- Pictorial representation.
- Alcohol swabs.
- Sterile lancets.

REF	302110025 (1T)	302110010	302110025	302110050	302110100
	25 X 1T	10	25	50	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 40°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 40°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 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 **DUO CHECK PT™** 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

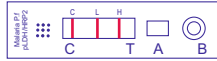
1. Wear gloves.
2. Collect blood by standard venipuncture procedure into a tube containing the correct anticoagulant (EDTA, heparin, citrate, CPDA or oxalate).
3. Mix the tube gently.
4. Transfer 5 µl of whole blood in the rectangular well (marked "A") of the cassette using a precision pipette.

- Perform steps 12–16 of the previous section ("Capillary whole blood from finger prick").

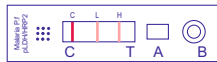
INTERPRETATION OF RESULTS



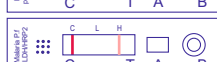
NEGATIVE for P.f malaria: A colored band appears in the control window 'C'.



POSITIVE for HRP-2 and pLDH of P.f malaria : In addition to the control band, a distinct colored band also appears at H and L.



POSITIVE for HRP-2 of P.f Malaria : In addition to the control band, a distinct colored band also appears at H.

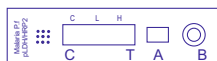


POSITIVE for pLDH of P.f Malaria : In addition to the control band, a distinct colored band also appears at L.

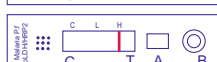


The test band area marked as 'H' represents the presence or absence of P.f HRP-2, and the test band area marked as 'L' represents the presence or absence of P.f pLDH in the specimen. Appearance of coloured band of any intensity (faint to dark) at H and/or L should be considered as positive result for *P. falciparum* malaria.

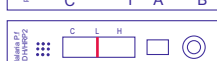
INVALID :



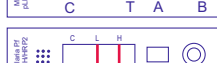
The test should be considered invalid if no coloured band appears on the device.



The test should be considered invalid if a coloured band appears only at H.



The test should be considered invalid if a coloured band appears only at L.



The test should be considered invalid if coloured band appears only at H and L.

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

Diagnostic Sensitivity and Specificity:

- In an internal study, a panel of 150 specimens whose results were earlier confirmed with microscopy were tested with **DUOCHECK Pf™**. The results obtained are as follows:

Specimen type	Total nos. of specimens tested	DUOCHECK Pf™			Sensitivity* %	Specificity* %
		Pf Positive	pLDH Positive	Negative		
Malaria Negative	100	0	0	100	-	100% (99.95% to 100%)
Pf and pLDH Positive	25	25	25	0	100% (99.16% to 100%)	-
Pv Positive	25	0	0	25	-	100% (99.16% to 100%)

*Relative Sensitivity and Specificity at 95% confidence intervals.

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

Possible Interferences:

DUOCHECK PF™ was tested using a variety of potentially interfering substances as given:

A) Pathogens: e.g. HIV(1 and 2), HCV, Measles IgM Plasma/Serum, Rf positive plasma, Hama positive serum, Leishmania positive Plasma, Schistosomiasis Sp. Patient sample, Ana positive 1, Chagas Antibody positive plasma, TBE positive plasma, Toxoplasma IgG plasma, Toxoplasma IgM positive, Multi Pregnant positive specimen, Multiple blood transfusion.

Precision: Reproducibility and repeatability studies (inter-assay and inter-lot) were carried out using a number of malaria-negative, *P. vivax* (Pv.) positive specimens, and *P. falciparum* (Pf. HRP-2/Pf. pLDH) positive specimens at strong, low-positive, and limit of detection (LoD) concentrations. **DUOCHECK PF™** generated results indicating 100% reproducibility and repeatability.

Based on these results, as well as in-house data, **DUOCHECK PF™** is a highly sensitive and specific test for the diagnosis of malaria caused by *P. falciparum*.

LIMITATIONS OF THE TEST

(1) As with all diagnostic tests, the results must always be correlated with clinical findings. (2) The results of test are to be interpreted within the epidemiological, clinical and therapeutic context. When it seems indicated, the parasitological techniques of reference should be considered (microscopic examination of the thick smear and thin blood films). (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. **DUOCHECK PF™** uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of these interferences. (5) **DUOCHECK PF™** is for detection of *P. falciparum* malarial infection. However, a negative test result does not rule out the possibility of infection with *P. vivax*, *P. ovale*, *P. knowlesi* and *P. malariae*. (6) Since Pf. pLDH is less sensitive marker for detection of *P. falciparum*, compared to Pf. HRP-2 marker, in Pf. HRP-2 gene deleted cases it may give negative results in very early stage of infection cases. (7) Since Pf. HRP-2 persists for upto a fortnight even after successful therapy, a positive test result does not indicate a failed therapeutic response. For therapeutic response Pf. pLDH band to be observed. If the reaction of the test band of Pf. HRP-2 and Pf. pLDH remains positive post treatment, the possibility of a resistant strain of malaria has to be considered. (8) If the reaction of the test remains positive with the same intensity after 5-10 days, post treatment, the possibility of a resistant strain of malaria has to be considered. (9) In *P. falciparum* malaria infection, Pf. HRP-2 is not secreted in gametogony stage. Hence in "Carriers", the "H" band may be absent. However, "L" band may appear since pLDH is produced and persist in gametogony stage also. (10) 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.

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) Bredu et al. Prevalence of *Plasmodium falciparum* parasites with pfhrp2 exon 2 gene deletion in symptomatic malaria patients across Ghana in 2021, Malaria Journal (2025) 24:170 <https://doi.org/10.1186/s12936-025-05419-1>. (6) Prosser et al. *Plasmodium falciparum* Histidine-Rich Protein 2 and 3 Gene Deletions in Strains from Nigeria, Sudan, and South Sudan. Emerging Infectious Diseases. 2021 Feb;27(2):471-479. doi: 10.3201/eid2702.191410. (7) Data on file : Orchid Biomedical Systems.

SYMBOL KEYS

	Temperature Limitation		Manufacturer		Device		This side up
	Use by		Consult Instructions for use		Contains sufficient for <n> tests		Do not reuse
	Date of Manufacture		Catalogue Number		Clearing Buffer		
	Batch Number / Lot Number		In vitro Diagnostic Medical Device		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.

Website: www.tulipgroup.com Email: sales@tulipgroup.com

Tel. : (0832) 2458546, (0832) 2458547

PI/30211/0826/VER-02
Date of issue: 2026-08