CE

paracheck® Pf

RAPID TEST FOR *P. FALCIPARUM* MALARIA (Ver.3)

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

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.
paracheck 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

paracheck 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

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.
   3. PIPETTE Disposable 5µl specimen applicator.
B. BUF Clearing buffer containing surfactant and preservative in a dropper bottle.
C. Instructions for Use.
D. Pictorial representation.
E. Alcohol swabs.
F. Sterile lancets.

REF	302030001	302030005	302030010	302030025	302030025(1T)	302030100
Σ	1	5	10	25	25 x 1	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 (0.1%), 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.

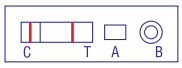
16. Read test results after a minimum of 20 minutes but no later than 30 minutes. Use a good light source when reading the test results.

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

Diagnostic Sensitivity And Specificity:

1. In an internal study, a panel of 498 specimens whose results were earlier confirmed with microscopy were tested with **paracheck Pf®**. The results obtained are as follows:

Specimen type	Total no. of specimens tested	paracheck Pf®		Sensitivity * %	Specificity* %
		Positive	Negative		
Malaria negative	210	2	208	-	99.05%
P. vivax positive	114	0	114	-	100%
P. falciparum positive	154	153	1	99.35%	-
RF positive (Malaria Negative)	20	0	20	-	100%

2. In an independent study, 192 whole blood specimens of febrile patients whose results were confirmed by microscopy were tested with **paracheck Pf®**. The results obtained are as follows:

Specimen type	Total no. of specimens tested	paracheck Pf®	
		Positive	Negative
Malaria negative	96	0	96
P. vivax positive	40	1	39
P. falciparum positive	50	49	1
P. vivax & *P. falciparum* positive (mixed infection)	6	6	0

paracheck Pf® was found to be 98.2% sensitive and 99.3% specific to *P. falciparum* malaria against microscopy.
* Relative Sensitivity and Specificity at 95% confidence intervals.

Possible Interferences:

paracheck Pf® was tested using a variety of potentially interfering substances as given:

a) Endogenous components: Substances such as bilirubin (direct, total), creatinine, triglycerides, uric acid, lipase proteins

and others at high physiological levels.

b) Exogenous components: substances such as anti-malarial drugs, antibiotics, anti-inflammatory drugs at high therapeutic concentrations.

c) Pathogens: e.g. HIV (1 and 2), HBV, HCV, *M. tuberculosis*, *S. typhi* and others. All specimens tested generated negative results in **paracheck Pf®**.

Precision:

Reproducibility and Repeatability studies (inter-assay and inter lot) were carried out using a number of malaria negative and Pv. positive specimens; and of strong, low positive and limit of detection Pf. positive specimens. **paracheck Pf®** generated results indicating 100% reproducibility and 100% repeatability.

From the above results and the results of in house data, **paracheck 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 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.

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	Σ	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		

Manufactured by:

Orchid Biomedical Systems

A Division of Tulip Diagnostics (P) Ltd.

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EC REP

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0522/VER-05
Date of issue:2023-01