Size: 274mm x 218mm

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paracheck® Pf

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

DIPSTICK

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 for the diagnosis of falciparum malaria in individuals with signs and symptoms consistent with malaria infection. The test is intended for healthcare professionals at clinical setup and point of care sites. The test is also suitable for detection of *P. falciparum* Malaria in neonates and pregnant women.

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 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 sample flows through the membrane assembly of the dipstick after placing into the clearing buffer tube, the colored Agglutinating Sera for HRP-2-colloidal gold conjugate complexes the HRP-2 in the lysed sample. 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. DIPSTICK : 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 sample applicator.

B. BUF : Clearing buffer containing surfactant and preservative in a dropper bottle.

C. Package insert.

REF	302040001	302040005	302040010	302040025	302040100
Σ	1	5	10	25	100

MATERIALS REQUIRED BUT NOT PROVIDED

A. Calibrated micro pipette capable of delivering 5 µl sample accurately. (Optional)
B. Clean (12 x 75) mm test tube.
C. Timer.
D. Disposable gloves.
E. Biohazard Waste container.

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.

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NOTES

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 dipstick 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 sample 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° 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 samples must be mixed gently prior to testing. Hemolysed, clotted or contaminated blood samples 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. In case the pouch has been stored at 2°C To 8°C, allow atleast 30 minutes for the dipstick to come to room temperature.
3. Open the pouch and retrieve the dipstick (taking care not to touch the membrane area), sample applicator and desiccant. Check the colour of the desiccant. It should be blue. If it has turned colourless or pink discard the dipstick and use another dipstick. ***Once opened, the dipstick must be used immediately.***
4. Label the dipstick with sample identity.
5. Tighten the vial cap of the clearing buffer provided with the kit in the clockwise direction to pierce the buffer bottle nozzle.
6. Evenly mix the anti coagulated blood sample by gentle swirling. Dip the sample applicator into the sample. Ensuring that an applicator full of blood is retrieved, blot the blood so collected on to the sample pad just below the arrows on the dipstick (This delivers approximately 5µl of the whole blood specimen).

   OR

   In case finger prick blood is being used, touch the sample applicator to the blood on the finger prick. Ensuring that an applicator full of blood is retrieved, immediately blot the specimen onto the sample pad just below the arrows on the dipstick (Care should be taken that the blood sample has not clotted and the transfer to the sample pad is immediate).

   OR

   Alternatively, 5 µl of the anti coagulated or the finger prick specimen may be delivered to the sample pad just below the arrows using a micro pipette.

   NOTE: Ensure that the blood from the sample applicator has been completely taken up by the sample pad.
7. Immediately dispense **four drops** of the clearing buffer into a clean 12 x 75 mm test tube by holding the plastic buffer bottle vertically.
8. Place the dipstick with the sample into the tube, with the arrows on the dipstick pointing downward and ensuring that the buffer level is below the blood sample for the entire duration of the test.
9. At the end of 20 minutes, read the results as follows:

NEGATIVE for *P. falciparum* malaria : A colored band appears on the dipstick.

POSITIVE for *P. falciparum* malaria : Two distinct colored bands appear on the dipstick.

INVALID : The test should be considered invalid if no colored band appears on the dipstick. The test should also be considered invalid if a colored band appears only at the test region and not at the control region. In such cases, repeat the test with a new dipstick, 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 samples.

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PERFORMANCE CHARACTERISTICS

Diagnostic Sensitivity And Specificity:

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

Sample type	Total no. of samples 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	20	0	20	-	100%

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

Sample type	Total no. of samples 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) Pathogenic micro-organisms: micro-organisms such as HIV (1 and 2), HBV, HCV, M. tuberculosis, S. typhi and others. All samples 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 samples; and of strong, low positive and limit of detection Pf. positive samples. **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 *falciparum* malaria.

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 sample 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 sample 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. In case the test needs to be used to monitor success of therapy, testing is advised only from 15 days after the completion of therapy.
8. Do not interpret the test results beyond 30 minutes.

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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

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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	DIPSTICK — Dipstick	Contains sufficient for <n> tests
Use by	Consult Instructions for use	PIPETTE — Disposable Plastic Sample 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

CMC Medical Devices & Drugs S.L., Spain.

0817/VER-02