CE

paramax-3™

RAPID TEST FOR MALARIA
Pan / Pv / Pf
DEVICE

INTENDED USE

paramax-3™ is a rapid, qualitative, two site sandwich immunoassay utilizing whole blood for the detection of *P.falciparum* specific histidine rich protein-2 (Pf. HRP-2), *P.vivax* specific pLDH and Pan malaria specific pLDH. The test can be used for the specific detection of *P. falciparum* and *P.vivax* malaria, differentiation of other malarial species and for the follow up of antimalarial therapy.

SUMMARY

Four species of the Plasmodium parasites are responsible for malaria infections in human viz. *P. falciparum, P.vivax, P.ovale* and *P.malariae*. Of these, *P. falciparum* and *P.vivax* are the most prevalent. Early detection and differentiation 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. As the course of treatment is dependent on the species, differentiation between *P. falciparum* and *P.vivax* is of utmost importance for better patient management and speedy recovery.

In **paramax-3**™ the detection system for *P. falciparum* malaria is based on the detection of *P. falciparum* specific histidine rich protein-2 (Pf. HRP-2) which is a water soluble protein that is released from parasitised erythrocytes of infected individuals. The detection system of *P.vivax* is based on the presence of *P.vivax* specific pLDH. Further the detection of other malarial infections such as *P.ovale* and *P.malariae* is achieved through the Pan malaria specific pLDH.

Since pLDH is a product of viable parasites, the Pan band may also be used to monitor course of effective antimalarial therapy.

paramax-3™ detects the presence of *P. falciparum* specific Pf. HRP-2, *P.vivax* specific pLDH and Pan specific pLDH in whole blood specimen and is a sensitive and specific test for the detection of all malaria species, differentiation for *P. falciparum* and *P.vivax* and monitoring successful antimalarial therapy.

PRINCIPLE

paramax-3™ 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 device after addition of the clearing buffer, the colored colloidal gold conjugates of the Agglutinating sera for HRP-2 and Agglutinating sera for Pan malaria specific pLDH complexes the HRP-2 / corresponding pLDH 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 and / or Agglutinating sera for *P. vivax* specific pLDH antibody and / or Agglutinating sera for Pan malaria specific pLDH coated on the membrane leading to formation of a pink-purple colored band in the respective regions which confirms a positive test result. Absence of a colored band in the test region indicates a negative test result for the corresponding antigen. The unreacted conjugate along with the rabbit globulin-colloidal gold 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 pink-purple band. The control band formation is based on the 'Rabbit / 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

paramax-3™ kit contains:

A. Individual pouches, each containing:
   1. DEVICE : Membrane assembly pre-dispensed with Agglutinating sera for HRP-2 - colloidal gold conjugate, Agglutinating sera for Pan malaria specific pLDH -colloidal gold conjugate, rabbit globulin-colloidal gold conjugate, Agglutinating sera for HRP-2, Agglutinating sera for *P. vivax* specific pLDH, Agglutinating sera for Pan malaria specific pLDH and Agglutinating sera for rabbit globulin at the respective regions.
   2. Desiccant pouch.
   3. PIPETTE : Disposable Plastic Sample Applicator.

B. BUF : Clearing buffer in a dropper bottle.

C. Package Insert.

REF	503020001	503020005	503020010	503020025
Σ	1	5	10	25

OPTIONAL MATERIAL REQUIRED

Calibrated micropipette capable of delivering 5µl sample accurately.

STORAGE AND STABILITY

The sealed pouches in the test kit & the kit components may be stored between 4°C to 30°C till the duration of the shelf life as

indicated on the pouch/carton. DO NOT FREEZE. After first opening of the clearing buffer bottle, it can be stored between 4°C to 30°C for the remaining duration of its shelf life.

NOTES

1. Read the instructions carefully before performing the test.
2. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use only.
3. Do not use the kit beyond expiry date and do not re-use the test device.
4. Do not intermix the reagents from different lots.
5. 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.
6. Handle all specimens as potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infective material.
7. 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.

SPECIMEN COLLECTION AND PREPARATION

Fresh anti coagulated whole blood should be used as a test sample. EDTA or CPDA or Heparin or Oxalate or Tri-sodium Citrate can be used as suitable anticoagulants. The specimen should be collected in a clean glass or plastic container. If immediate testing is not possible then the specimen may be stored at 2°C to 8°C for up to 72 hours before testing. 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.

TESTING PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the **paramax-3**™ kit components to room temperature before testing.
2. Open the pouch and retrieve the device, sample applicator and desiccant pouch. Check the color of the desiccant. It should be blue, if it has turned colorless or pink, discard the device and use another device. ***Once opened, the device must be used immediately.***
3. Label the test device with patient's identity.
4. Tighten the cap of the clearing buffer bottle provided with the kit in the clockwise direction to pierce the dropper bottle nozzle.
5. 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 in the sample port 'A'. (This delivers approximately 5µl of the whole blood specimen).

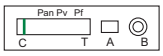
   OR

   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 in the sample port 'A'. (Care should be taken that the blood sample has not clotted and the transfer to the sample port is immediate).

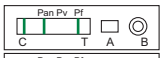
   OR

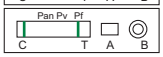
   Alternatively, 5µl of the anti coagulated or finger prick specimen may be delivered in the sample port 'A' using a micro pipette.

   NOTE : Ensure that the blood from the sample applicator has been completely taken up at the sample port 'A'.
6. Immediately dispense **two drops** of clearing buffer into buffer port 'B', by holding the buffer bottle vertically.
7. Read the results at the end of **20 minutes** as follows :

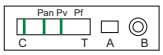


NEGATIVE for malaria: Only one pink-purple band appears at the control region 'C'.

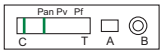




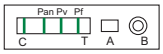
POSITIVE for *P. falciparum* or mixed infection with Pf or mixed infection with Pf other than Pv (*P.malariae* or *P.ovale*): In addition to the control band, two pink-purple bands appear at regions 'Pf' and 'Pan' in the test window 'T'.
In addition to the control band, appearance of One pink-purple band at 'Pf' region, should also be considered as Pf positive.



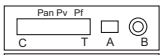
POSITIVE for *P.vivax* malaria: In addition to the control band, two pink-purple bands appear at regions 'Pv' and 'Pan' respectively.



POSITIVE for Other species: In addition to the control band, a pink-purple band appears only at region 'Pan'.



POSITIVE for Mixed infection: In addition to the control band, three pink-purple bands appear at regions 'Pf', 'Pv' and 'Pan' respectively.



INVALID RESULT: The test should be considered invalid if no bands appear on the device. The test should also be considered invalid if only test bands (Pan and/or Pv and/or Pf) appear and no control band appears. Repeat the test with a new device ensuring that the test procedure has been followed accurately.

PERFORMANCE CHARACTERISTICS

In an in-house study, a panel of 251 samples whose results were earlier confirmed with microscopy were tested with **paramax-3**™. The results obtained are as follows:

Sample	Total No. of samples tested	paramax-3™		Sensitivity (%)	Specificity (%)
		Positive	Negative		
P. falciparum positive	16	16	0	100	-
P. vivax positive	25	25	0	100	-
Malaria negative	210	0	210	-	100

LIMITATIONS OF THE TEST

1. As with all diagnostic tests, the test result 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 sample can lead to erroneous analyte detection in immunoassay, has been reported in various studies. **paramax-3**™ uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of these interferences.
5. In case of infection due to *P.vivax* or *P.falciparum*, or due to mixed infection by these species, the 'Pan' malaria band will also be positive. Hence, differentiation of infection due to *P.ovale* or *P.malariae* cannot be done.
6. While monitoring therapy, 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.
7. Usually, the 'Pv' and 'Pan' bands turn negative after successful anti malarial therapy. However, since treatment duration and medication used affect the clearance of parasites, the test should be repeated after 5-10 days of start of treatment.
8. In *P.falciparum* malaria infection, HRP-2 is not secreted in gametogony stage. Hence, in "Carriers", the HRP-2 band may be absent.
9. HRP-2 levels, post treament persists upto 15 days, the 'Pan' band can be used to monitor success of therapy in *P.falciparum* malaria cases.
10. In a few cases, where the HRP-2 band is positive and the 'Pan' malaria band is negative, it may indicate a case of post treatment malaria. However, such a reaction pattern may also be obtained in a few cases of untreated malaria. Retesting after 2 days is advised, in such cases.
11. Do not interpret the test results beyond 30 minutes.
12. As the sensitivity of HRP-2 detection in Pf Malaria is higher than that of pLDH (Pan), it is possible that in Pf Malaria positive cases only the 'Pf' band will appear without the appearance of 'Pan' band.
13. In Pf infection cases that has been resolved through anti-malaria treatment, the Pan (pLDH) would turn negative while residual HRP-2 would continue to circulate in the patient for a week to 10 days giving a band at 'Pf' region.

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 a Malarial Histidine-rich Protein (Pf. HRP II) from *Plasmodium falciparum*-infected Erythrocytes.J. Cell Biol., 103, 1269-1277.
2. 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.
3. 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.
4. Piper, R. C., et al., (1999) Immuno-capture diagnostic assays for malaria utilizing *Plasmodium* Lactate Dehydrogenase (pLDH) Am. J. Trop. Med. Hyg. 60(1) 109-118.
5. Hunte-Cooke A., et al., (1999) Comparison of a Parasite Lactate Dehydrogenase-based Immunochromatographic Antigen Detection assay (OptiMAL®) with Microscopy for the Detection of Malaria Parasites in Human Blood Samples. Am J.Trop Med 60(2). 173-176.
6. Quintana M., et al., (1998) Malaria diagnosis by dipstick assay in a Honduran Population with coendemic *Plasmodium falciparum* and *Plasmodium vivax*. Am. J. Trop. Med. Hyg. 59(6) 868-871.
7. Palmer, C. J., (1998) Evaluation of OptiMal test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum*. J. Clin Microbiol. 36(1) 203-206.
8. Moody A., et al., (2000) Performance of the OptiMAL® malaria antigen capture dipstick for malaria diagnosis and treatment monitoring. British Journal of Hematology, 109, 1-5.
9. Data on file: Viola Diagnostic Systems.

SYMBOL KEYS

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

Manufactured by:

Viola Diagnostic Systems

A Division of Tulip Diagnostics (P) Ltd.

Plot No. 33, Sector-3, I.I.E. SIDCUL, Pantnagar, U. S. Nagar, Uttarakhand - 263 153, 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

EC REP

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

1223/VER-05