



REF	503230025(1T)	503230010	503230025
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Rapid Test for Malaria Pv pLDH / P.f pLDH+P.f HRP2

DEVICE

INTENDED USE

CombiVIEW™-Pv/Pf is a rapid, qualitative, two site sandwich immunoassay utilizing whole blood for the detection of *P. vivax* specific pLDH and *P. falciparum* specific plasmodium lactate dehydrogenase and *P. falciparum* specific histidin rich protein-2 (P.f pLDH+P.f HRP-2 combined). The test can also be used for specific detection and differentiation of *P. vivax* malaria and *P. falciparum* malaria in areas with high rates of mixed infections.

The test is intended for professional use at clinical and point of care sites in suspected cases of malaria infection.

SUMMARY

Five species of the Plasmodium parasites are responsible for malarial infections in human viz. *P. falciparum*, *P. vivax*, *P. ovale*, *P. knowlesi* and *P. malariae*. Of these *P. falciparum* and *P. vivax* are considered the "Big Two" due to incidence of cerebral malaria and drug resistance associated with *P. falciparum* malaria, and high rate of infectivity and relapse associated with *P. vivax*. 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.

Moreover, It has been observed that emergence of *P. falciparum* parasites with deletions in the Pf HRP-2 and Pf HRP-3 genes in most malaria-endemic regions poses a significant biological threat to falciparum 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 Pf pLDH detection for diagnosis of *P. falciparum* malaria.

In **CombiVIEW™-Pv/Pf** detection system for *P. vivax* malaria is based on presence of *P. vivax* specific Plasmodium lactate dehydrogenase (pLDH) released from parasitized erythrocytes of vivax infected individuals and the detection system for *P. falciparum* malaria is based on the detection of *P. falciparum* specific histidine rich protein-2 and *P. falciparum* specific Plasmodium lactate dehydrogenase (Pf HRP-2+Pf pLDH combined), released from parasitized erythrocytes of falciparum infected individuals.

PRINCIPLE

CombiVIEW™-Pv/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 colloidal gold conjugates of the Agglutinating Sera for HRP-2, Agglutinating Sera for pLDH complexes the HRP-2, Pf pLDH/ Pv pLDH in the lysed specimen. This complex moves further on the membrane to the test region where it is immobilized by the Agglutinating Sera for *P. vivax* Malaria specific pLDH and Agglutinating Sera for P.f HRP-2 and P.f pLDH coated on the membrane leading to formation of pink-purple colored band/s which confirms a positive test result. A band will appear under Pf at the test region in falciparum positive specimens, while a band will appear under Pv in vivax malaria positive specimens. Appearance of band under Pf as well as Pv in the test region suggests a mixed infection.

Absence of colored band/s 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 immobilized by 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 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

CombiVIEW™-Pv/Pf 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 pLDH - colloidal gold conjugate, rabbit globulin - colloidal gold conjugate, Agglutinating Sera for P.f pLDH+P.f HRP2, Agglutinating Sera for *P. vivax* Malaria specific pLDH and Agglutinating Sera for Rabbit globulin at the respective regions

2. Desiccant pouch.

B. **INVERTED CUP** Disposable Plastic Specimen Applicator.

C. **BUF** Clearing buffer in a dropper bottle.

D. Instructions for use.

E. Pictorial instruction for use.

F. Alcohol Swab-70% Isopropyl alcohol.

G. Sterile lancets.

Product codes	REF	503230025(1T)	503230010	503230025
Pouch Sealed tests		25 x 1T (25 single kit test/kit)	10	25
Disposable Plastic Specimen Applicator		25	10	25
Clearing buffer bottles		25 x 1.0ml	01 x 3.0ml	01 x 4.0ml
Alcohol swabs		25	10	25
Sterile lancets		25	10	25
Instructions for use		25	01	01
Pictorial instructions for use		25	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 venipuncture).

Additional alcohol swabs (if any included in the kit are found dry) and additional sterile lancets (if any included in the kit have the sterility seal broken).

STORAGE AND STABILITY

The sealed pouches in the test kit & the kit components may be stored between 2°C to 40°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 2°C to 40°C for the remaining duration of its shelf life.

WARNINGS

Read the instructions carefully before performing the test.

For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use.

The test is for aiding in diagnosis of malaria infection and not for screening which requires confirmation.

Do not use beyond expiry date.

Do not use components from different lots of the product.

The device, specimen applicator, alcohol swab and blood lancet are for single use only.

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.

Handle all specimens as potentially infectious.

Follow standard biosafety guidelines for handling and disposal of potentially infectious material.

Clearing buffer contains Sodium Azide(0.095%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing system and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build up in the plumbing.

SPECIMEN COLLECTION AND PREPARATION

For specimen collection, refer to pictorial instructions for use.

Fresh capillary/venous whole blood from finger prick / puncture should be used as a test specimen. However, fresh anti-coagulated venous whole blood may also be used as a test specimen. Using standard blood collection practices, collect venous whole blood into the commercially available anti-coagulant tube such as EDTA or CPDA or Heparin or Oxalate or Tri-sodium Citrate. If immediate testing is not possible then the specimen may be stored at 2°C to 8°C for upto 72 hours before testing and should be brought to room temperature (20°C to 30°C) before use on the test. Clotted, hemolysed or lipaemic whole blood specimens should not be used for performing the test.

TESTING PROCEDURE AND INTERPRETATION OF RESULTS

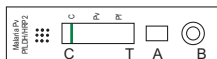
1. Bring the **CombiVIEW™-Pv/Pf** kit components to room temperature (20°C to 30°C) before testing.
2. Open the pouch and retrieve the device and desiccant pouch. Check the color of the desiccant. It should be blue, if it has turned colorless or pink, discard the device and use another device. **Once opened, the device must be used immediately.**
3. Label the test device with patient's identity.
4. Place the testing device on a flat horizontal surface.
5. Tighten the cap of the clearing buffer bottle provided with the kit in the clockwise direction to pierce the buffer bottle nozzle.
6. **Specimen application**

6.1 Venous whole blood: Evenly mix the anti-coagulated whole blood by gentle swirling. Dip the specimen applicator into the whole blood. Ensuring that an applicator full of blood is retrieved, immediately blot the blood so collected in the specimen port 'A'(This delivers approximately 5µl of the whole blood specimen).

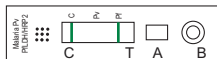
6.2 Capillary whole blood: Touch the specimen applicator to the whole blood on the finger prick. Ensuring that an applicator full of blood is retrieved, immediately blot the blood so collected in the specimen port 'A'(Care should be taken that whole blood specimen is not clotted and transfer to the specimen port is immediate).

Note: It is recommended to use specimen applicator provided with the kit. Alternatively, 5µl of the anti-coagulated venous whole blood/capillary finger-prick whole blood specimen may be delivered in the specimen port 'A' using a calibrated micropipette. Ensure that the whole blood from the specimen applicator/calibrated micropipette has been completely taken up at the specimen port 'A'.

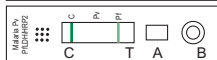
7. Immediately dispense **two drops** of clearing buffer into buffer port 'B' holding the buffer bottle vertically and switch on the timer. To avoid contamination of clearing buffer bottle, do not touch the buffer port 'B' with the tip of clearing buffer bottle.
8. Read the results at the end of **20 minutes** as follows:



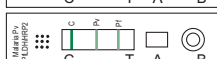
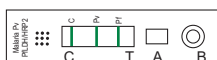
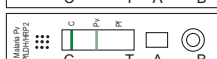
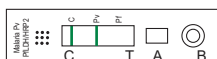
NEGATIVE for malaria: A colored band appears in the control window 'C'.



POSITIVE for Pf malaria : In addition to the control band, a distinct colored band also appears at Pf.



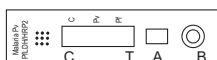
POSITIVE for Pv Malaria : In addition to the control band, a distinct colored band also appears at Pv.



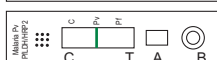
POSITIVE for Pf and Pv Malaria : In addition to the control band, a distinct colored band also appears at Pf and Pv.

Appearance of coloured bands of any intensity (Faint or Dark) at Pf and Pv should be considered as positive results for *P. falciparum* and *P. vivax* malaria.

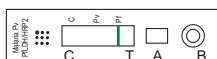
INVALID :



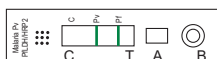
The test should be considered invalid if no coloured band appears on the device.



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



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



The test should be considered invalid if coloured band appears only at Pf and Pv.

In such cases, repeat the test with a new device, ensuring that the test procedure has been followed accurately.

PERFORMANCE CHARACTERISTICS

Internal Evaluation:

In an in-house study, a panel of 170 venous whole blood specimens whose results were earlier confirmed with microscopy were tested with **CombiVIEW™-Pv/Pf**. The results obtained are as follows:

Specimens	Total Nos. of Specimens tested	CombiVIEW™-Pv/Pf		Sensitivity 95% (CI)	Specificity 95% (CI)
		Positive	Negative		
<i>P. falciparum</i>	25	25	0	100% (86.28% to 100%)	-
<i>P. vivax</i>	25	25	0	100% (86.28% to 100%)	-
Malaria Negative	120	0	120	-	100% (96.97% to 100%)

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. **CombiVIEW™-Pv/Pf** uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of these interferences. (5) **CombiVIEW™-Pv/Pf** is 100% sensitive to *P. falciparum* and *P. vivax* malaria. However, a negative test result does not rule out the possibility of infection with *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) In case of infection with *P. vivax* usually, the 'Pv' bands can be employed for monitoring success of 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) 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 'Pf' band may be absent. (10) Since Pf. HRP-2 persists for upto a fortnight even after successful therapy, a positive test result does not indicate a failed therapeutic response. 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. (11) The 'Pv' band can be used for monitoring success of anti malarial therapy, in case of stand alone *P. vivax* infection. For monitoring success of anti malarial therapy in case of stand alone *P. falciparum* infection or mixed infection, employing a Pan specific pLDH based system is recommended after 5-10 days of initiation of the chemotherapeutic agent. (12) 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 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) Bredu et al. Prevalence of *Plasmodium falciparum* parasites with pfrp2 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>. (10) 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. (11) Data on file: Zephyr Biomedicals.

SYMBOL KEYS

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



Manufactured by:

Zephyr Biomedicals

A Division of Tulip Diagnostics (P) Ltd.

M 46-47, Phase III B, 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