SBio Malaria Pv/Pf Test

Rapid test for Malaria Pv/Pf

REF	90300025
Σ	25 T

CE

Temperature Limitation	Manufacturer	PIPETTE — Disposable Plastic Sample Applicator	EC REP — Authorised Representative in the European Community	Xn NaN_3 R22 S23-46-61 Harmful if swallowed. Do not breathe vapour. If swallowed, seek medical advice immediately and show this container or label. Avoid release to the environment. Refer to special instructions.
Use by (Last day of stated month)	Consult Instructions for use	DEVICE — Device	BUF — Assay Buffer	
Date of Manufacture	REF — Catalogue Number	Σ — Contains sufficient for <n> tests	Do not reuse	
LOT — Batch Number/ Lot Number	IVD — *In vitro* Diagnostic Medical Device	This side up	Malaria Pv/Pf — Rapid test for malaria Pv/Pf	

INTENDED USE

SBio Malaria Pv/Pf Test 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) and *P.vivax* specific pLDH. 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.

SUMMARY

Four species of the Plasmodium parasites are responsible for malarial infections in human viz. *P. falciparum*, *P.vivax*, *P.ovale* 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.

In SBio Malaria Pv/Pf Test**,** 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 for *P.vivax* malaria is based on presence of *P.vivax* specific pLDH.

PRINCIPLE

SBio Malaria Pv/Pf Test utilizes the principle of immunochromatography. As the test sample flows through the membrane assembly of the device after addition of the clearing buffer, the colored colloidal gold conjugates of monoclonal anti-Pf. HRP-2 (IgG) antibody and monoclonal anti Pan specific pLDH antibody complexes the HRP-2/ pLDH in the lysed sample. This complex moves further on the membrane to the test region where it is immobilized by the anti vivax specific pLDH (monoclonal) antibody and / or the monoclonal anti-Pf. HRP-2 (IgM) antibody 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 samples, while a band will appear under Pv in vivax malaria positive samples. 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 anti rabbit antibodies coated on the membrane at the control region, forming a pink-purple band. The control band formation is based on the 'Rabbit / anti-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

SBio Malaria Pv/Pf Test kit contains:

A. Individual pouches, each containing:
   1. Device: Membrane assembly pre-dispensed with monoclonal anti-Pf. HRP-2 (IgG) antibody colloidal gold conjugate, monoclonal anti Pan specific pLDH antibody-colloidal gold conjugate, rabbit globulin colloidal gold conjugate, monoclonal anti-Pf. HRP-2 (IgM) antibody, monoclonal anti *P.vivax* specific pLDH antibody and anti rabbit antibody at the respective regions.
   2. Desiccant pouch.
   3. Pipette: Disposable 5µl Sample loop.

B. Buf: 0.1 M diSodium tetraborate, 1% Triton X-100, with 0.1% Sodium Azide.

C. Package Insert.

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 4-30°C till the duration of the shelf life as indicated on the pouch/carton. DO NOT FREEZE.

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 beyond expiry date.
4. Do not reuse the test device.
5. Do not intermix the reagents from different lots.
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 blood from finger prick / puncture should be used as a test specimen. However, fresh anti coagulated whole blood may also be used as a 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 the specimen may be stored at 2-8°C for upto 72 hours before testing. Clotted or contaminated blood samples should not be used for performing the test.

TESTING PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the SBio Malaria Pv/Pf Test kit components to room temperature before testing.
2. Open the pouch and retrieve the device, sample loop 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/specimen identity.
4. Tighten the vial cap of the clearing buffer 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 loop into the sample. Ensuring that a loop 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 loop to the blood on the finger prick. Ensuring that a loop 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).

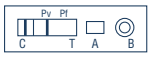
OR

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

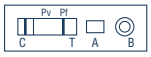
NOTE : Ensure that the blood from the sample loop has been completely taken up at the sample port 'A'.

6. Immediately dispense **two drops** of clearing buffer into buffer port 'B', by holding the plastic dropper bottle vertically.
7. Read the results at the end of **20 minutes** as follows:

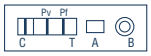
NEGATIVE for malaria : Only one pink-purple band appears in the control window 'C'.



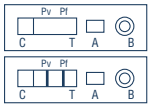
POSITIVE for *P.vivax* malaria : In addition to the control band, a pink-purple band also appears under the region marked ' Pv' in the test window 'T'.



POSITIVE for *P.falciparum* malaria : In addition to the control band, a pink-purple band also appears under the region marked 'Pf' in the test window 'T'.



POSITIVE for *P.falciparum* and *P.vivax* malaria : In addition to the control band, two pink-purple bands appear under the regions marked 'Pf' and 'Pv' in the test window 'T'.



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 (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 207 samples whose results were earlier confirmed with microscopy were tested with SBio Malaria Pv/Pf. The results obtained are as follows:

Sample	Total no. of samples tested	SBio Malaria Pv/Pf		Sensitivity (%)	Specificity (%)
		+ve	-ve		
Pf +ve	22	22	0	100	-
Pv +ve	17	17	0	100	-
Malaria -ve	168	0	168	-	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 sample can lead to erroneous analyte detection in immunoassay, has been reported in various studies. SBio Malaria Pv/Pf uses HETEROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of these interferences.
5. SBio Malaria 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* and *P. malariae*.
6. 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.
7. 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.
8. In *P. falciparum* malaria infection, Pf. HRP-2 is not secreted in gametogony stage. Hence in "Carriers", the 'Pf' band may be absent.
9. 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.
10. 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.
11. Do not interpret the test results beyond 30 minutes.

BIBLIOGRAPHY

1. Makler, M. T., et al., (1993) Parasite lactate assay as an assay for *Plasmodium falciparum* drug sensitivity. Am. J. Trop. Med. Hyg. 48(6), 739-741.
2. 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.
3. Srinivasan. S., et al., (2000) Comparison of blood-film microscopy, The OptiMAL dipstick, Rhodamine-123 fluorescence staining and PCR for monitoring antimalarial treatment. Annals of Tropical Medicine and Parasitology, 94(3) 227-232.
4. 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.
5. John, S. M., et al., (1998) Evaluation of OptiMAL, a dipstick test for the diagnosis of malaria. Ann. Trop. Med. Parasitol., 92, 621-622.
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.

Singapore Biosciences PTE Ltd.

11 Yishun Street 51, 304-23,The Criterion, Singapore 767971

EC REP

CMC Medical Devices & Drugs S.L., C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain

0620VER-01