VISITECT® MALARIA COMBO PAN / Pf Ref OD206

Rapid test for the determination of P. falciparum, Non falciparum or mixed infections in Whole Blood. Store at 4°C to 30°C. DO NOT FREEZE. For in-vitro diagnostic use only.

INTRODUCTION AND INTENDED USE

Four species of the Plasmodium parasites are responsible for malaria infections in humans: 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. The period of incubation, symptoms and duration of attack vary with the species and the individual's level of acquired immunity. Early detection and differentiation of malaria is therefore of paramount importance due to the incidence of cerebral malaria and associated drug resistance. **VISITECT MALARIA COMBO Pan / Pf.** determines malarial infection by the detection of pan malaria specific plasmodium Lactate Dehydrogenase (pLDH) released from parasitized red blood cells. Additionally **VISITECT MALARIA COMBO Pan / Pf.** determines specific infection by P.falciparum by the detection of P.falciparum specific histidine rich protein-2 (Pf HRP-2), a water-soluble protein that is released from parasitised erythrocytes of infected individuals and is species specific.

VISITECT MALARIA COMBO Pan / Pf. is a rapid, Point-of-Care, qualitative, two-site sandwich immunoassay for the determination of pLDH and Pf HRP-2 in whole blood samples. For professional use only.

PRINCIPLE OF THE TEST

VISITECT MALARIA COMBO Pan / Pf. is a rapid test for the detection of P.falciparum malaria, non P.falciparum malaria or mixed malaria infections that utilises the principle of Immunochromatography. As the test sample flows through the membrane assembly of the device, after addition of the diluent buffer, the anti - pLDH and the anti-Pf HRP-2 coloured colloidal gold monoclonal antibody conjugates complex with the pLDH and Pf HRP-2 if present in the lysed sample. This complex moves further along the membrane to the test region where it is immobilised by the anti - pLDH and anti-Pf HRP-2 monoclonal antibodies coated on the membrane, leading to formation of pink coloured lines, which confirms a positive test result.
Two bands will appear in the test region in falciparum positive samples, one band will appear in non falciparum positive samples. Absence of coloured lines in the test region indicates a negative test result.
The unreacted conjugate, unbound complex, if any, and the colloidal gold conjugated rabbit IgG moves further along the membrane and is subsequently immobilised by the goat anti-rabbit IgG coated on the membrane at the control region, forming a pink line. This control line serves to validate the test performance.

CONTENTS

Ref
OD206

25

Test	Device

Comprising of membrane assembly predispensed with anti - pLDH and anti-Pf HRP-2 colloidal gold conjugated monoclonal antibodies and colloidal gold conjugated rabbit IgG. Anti - pLDH and anti-Pf HRP-2 monoclonal antibodies on the Test line and goat anti-rabbit IgG on the Control line. Disposable 5µl sample applicator pipette. Desiccant bag.

Buf	7.5 ml

Diluent Buffer. Solution of Trizma-Base and Triton X-100

INSTRUCTION LEAFLET 1

PRECAUTIONS

VISITECT reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation. Do not ingest.

VISITECT MALARIA COMBO Pan / Pf. Diluent buffer contain 0.095% sodium azide as a preservative which may be toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive salts. On disposal, flush with large quantities of water.

STORAGE

Reagents must be stored at temperatures between 4°C to 30°C.

The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Expiry date is the last day of the month on the pouch and the kit label. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

DO NOT FREEZE DEVICE as this will cause irreversible damage.

SPECIMEN COLLECTION AND PREPARATION

Obtain a sample of venous blood from the patient and add to an EDTA, Heparin or Citrate plasma collection vial.

Samples may be used immediately or stored at 2°C to 8°C for up to 72 hours prior to testing.

Fresh whole blood samples may also be used with this kit. See Assay procedure for methodology.

REAGENT PREPARATION

Devices and samples should be brought to room temperature (20°C to 25°C) and mixed gently prior to use.

In case the pouch has been stored at 4°C to 8°C, allow at least 30 minutes for the device to come to room temperature. Check the colour of the desiccant. It should be blue. If it has turned colourless or faint blue, discard the device and use another device.

LIMITATIONS OF USE

The use of samples other than whole blood or EDTA, Heparin or Citrate whole blood have not been validated in this test.

There is no reuse protocol for this product.

A low or suspected positive result should be re-assessed. Diagnosis should not be made solely on the findings of one clinical assay. When making an interpretation of the test it is strongly advised to take all clinical data into consideration.

HRP – 2 is not secreted in the gametogony stage of **P.falciparum malaria infection therefore consideration of this aspect must be considered during interpretation.**

When present Pf HRP-2 persists for up to a two weeks (even after successful therapy), a positive test result does not therefore indicate a failed therapeutic response.

pLDH is a product of viable parasites therefore the pan band may be used to monitor anti malarial therapy. If the Pan line remains at the same intensity for 5 to 10 days post treatment resistance should be considered.

If the HRP – 2 line is positive but the Pan line is negative this may be the effect of successful treatment but the patient should be consulted and the test repeated after a two day period.

In the case of a mixed infection involving P.falciparum malaria and another malarial species both the Pan and Pf lines will be positive.

Most blood samples clear within running the test. However, in a few fresh samples and especially in stored samples, the background clearance may be delayed for 15-20 minutes more. In such cases it is strongly recommended to extend the reading time by another 15 minutes so that the results can be interpreted against a clear background.

ASSAY PROCEDURE

1. Open the pouch and remove the device. Once opened, the device must be used immediately.
2. Evenly mix the anti-coagulated blood sample by gentle swirling. Touch the sample applicator pipette to the surface of the blood in the sample container. Blot the blood so collected on to the sample pad in the sample well 'A'. (This delivers approximately 5µl of the whole blood specimen).

   OR

   In case finger prick blood is being used, touch the sample applicator pipette to the blood on the finger prick and immediately blot the specimen on to the sample pad in the sample well 'A'. (Care should be taken that the blood sample has not clotted and the transfer to the sample pad is immediate.

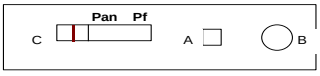
   OR

   Alternatively, 5µl of the anti-coagulated or finger prick specimen may be delivered to the sample pad in the sample well 'A' using a micropipette.

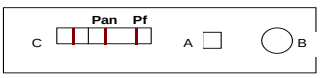
   NOTE: Ensure the blood from the sample applicator pipette has been completely taken up by the sample pad.
3. Dispense four drops of the diluent buffer into well 'B', by holding the plastic dropper bottle vertically.
4. At the end of 15 minutes, read the results.
   However ,if the background of the result window has not cleared within this time, wait for another 15 minutes before reading the results.

RESULTS AND INTERPRETATION

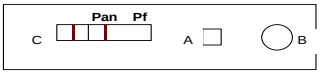
Negative: for malaria: Only one pink - purple coloured line appears in the control window 'C'.



Positive: for malaria P.falciparum or mixed infection: In addition to the control line, two distinct pink - purple coloured lines appear at "Pf" and "Pan" sections of the test window.



Positive: for malaria non – P.falciparum: In addition to the control line, a distinct pink - purple coloured line also appears in the "Pan" section of the test window.



The test should be considered invalid if no line appears. Repeat the test with a new device.

TROUBLESHOOTING

Use a separate disposable sample applicator pipette for each sample to prevent cross contamination.

Prior to the start of the assay bring all reagents to room temperature (20°C to 25°C).

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

EVALUATION DATA

Reproducability of **VISITECT MALARIA COMBO Pan / Pf.** is 100% (+/- one double dilution).

In a study, 251 samples whose results were earlier confirmed with expert microscopy were tested with **VISITECT MALARIA COMBO Pan / Pf** and the results obtained were as follows:

Sample Type	Total No. of Samples Tested	VISITECT Malaria Combo Pan / Pf		Sensitivity %	Specificity %
		+	-		
P.falciparum	16	16	0	100	-
P.vivax Positive	25	25	0	100	
Malaria Negative	210	0	210	-	100

REFERENCES

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. 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. 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.
6. 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.
7. 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.
8. 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.
9. John, S. M., et. al.,(1998) Evaluation of OptiMAL,a dipstick test for the diagnosis of malaria. Ann. Trop. Med. Parasitol., 92, 621-622. 1
10. 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. 11.Palmer, C. J.,((1998) Evaluation of OptiMal test for rapid diagnosis of Plasmodium vivax and Plasmodium falciparum . J. Clin Microbiol . 36(1) 203-206.
11. 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 .
12. Data on file, Omega Diagnostics Ltd.

Issue 1 Revised July 2004

OMEGA DIAGNOSTICS LTD.
Omega House, Hillfoots Business Village
Alva FK12 5DQ, Scotland, United Kingdom
odl@omegadiagnostics.co.uk
www.omegadiagnostics.com

AN ISO 9001 AND ISO 13485 CERTIFIED COMPANY