Ref: 5499

PALUTOP® +4 OPTIMA

Rapid test for the detection of the 4 *Plasmodium* in blood

1. INTENDED USE

PALUTOP®+4 OPTIMA is a simple and rapid test designed for the detection of the 4 *Plasmodium (Plasmodium falciparum (Pf), Plasmodium vivax (Pv), Plasmodium. malariae (Pm), Plasmodium ovale (Po))* in whole blood and venous blood. This test detects the 4 Plasmodium species, differentiates the *P. falciparum* and the *P. vivax* malaria and helps monitoring successful anti-malarial therapy.

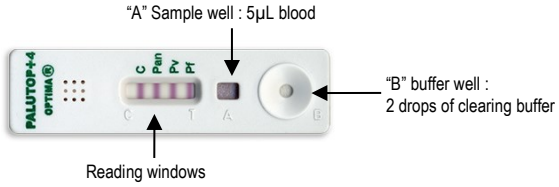
2. INTRODUCTION

Malaria (paludism) is caused by 4 Plasmodium species: *P. falciparum, P. vivax, P. ovale* and *P. malariae*. Of these, *P. falciparum* and *P. vivax* are the most prevalent species. A specie differentiation is essential, in particular for the *P. falciparum specie.* Early detection of *P. falciparum* malaria is of paramount importance due to incidence of cerebral malaria and drug resistance associated with it.

PALUTOP®+4 OPTIMA detects the presence of the water soluble HRP-2 protein (histidine-rich protein II) *P. falciparum* specific (Pf band Test), of pLDH (Lactate dehydrogenase) enzyme *P. vivax* specific (Pv band Test) and of pLDH enzyme common Plasmodium species *P. falciparum, P. vivax, P. malariae, P. ovale* (Pan band Test). Both pLDH isoform are produced by viable parasites.

3. TEST PRINCIPLE

PALUTOP®+4 OPTIMA is a chromatographic sandwich dye conjugate immunoassay. During the sample migration along the membrane, the monoclonal anti-Pf.HRP-2, and anti-pLDH antibodies labelled with colloidal gold particles, binds to the corresponding antigens (Pf HRP-2 protein, Pv LDH enzyme, pLDH enzyme) present in the specimen, which has been buffer lysed. The antibody-antigen complex binds to the Pf HRP-2 specific monoclonal antibody and/or to the Pv LDH specific monoclonal antibody and/or to the pLDH specific monoclonal antibody, respectively fixed on the Pf, Pv and Pan test band. Depending on the present antigens, the corresponding purple coloured bands (Pf, Pv, Pan) appear. The purple coloration of the C control band demonstrates that the test is functioning correctly.



4. MATERIAL PROVIDED

The PALUTOP®+4 OPTIMA kit contains the material required for the realization of 10 tests:

- 10 devices, packed in individual pouch containing :
  - 1 sample loop 5 µL,
  - 1 desiccant bag.
- 1 clearing buffer vial DIL, of 3 mL,
- 1 bilingual instructions for use

5. MATERIAL REQUIRED BUT NOT PROVIDED

- Timer with alarm
- Sampling lancets
- Antiseptic solution
- Laboratory pipette capable of delivering 5 µL accurately.

6. STORAGE AND STABILITY

- For in vitro diagnostic use only. For professional use.
- Use the kit before the expiration date.
- Store the kit in a dry area, at a temperature between 4°C and 30°C until expiry date mentioned on the kit labels. The kit (device and clearing buffer) however can be stored, without alteration of the life and qualities of the product, between -20°C and 45°C for 4 weeks.
- After opening, the buffer vial can be stored until the expiry date mentioned on the vial label.
- After opening the pouch, the device must be immediately used within one hour maximum.
- Do not freeze the kit.

7. PRECAUTIONS

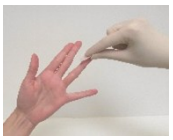
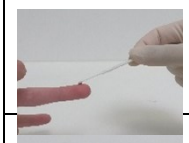
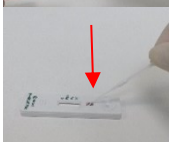
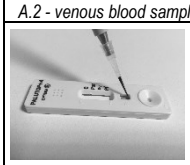
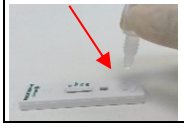
- Use the test, by respecting *in vitro* conditions, before the expiration date.
- Do not eat, smoke or drink while realizing the test.
- Use disposable laboratory gloves during the test procedure. Consider the clinical samples as potentially infectious and manipulate them according to the current good clinical practices and recommendations.
- For best results, follow strictly the test procedure and storage instructions.
- To prevent formation of condensation, do not open the device pouch until it has reached room temperature.
- Do not manipulate used devices or buffers again. Proceed to their elimination by following the procedure reserved to potentially dangerous garbage.
- Clean up the splash by using an appropriate disinfectant.

8. SPECIMEN COLLECTION AND HANDLING

- Fresh anti coagulated whole blood should be used as a test sample and Heparin, Oxalate or EDTA can be used as suitable anticoagulant tube. The specimen should be collected in a clean glass or plastic container. Fresh blood from finger prick / puncture may also be used as a test specimen. Clotted or contaminated blood samples should not be used for performing the test.
- Operate the test after collecting. The sample can be store 7 hours at ambient temperature or up to 72 hours between 2°C and 8°C before testing. For long-term storage, freeze the sample at -20°C. Repeated freezing and thawing of the sample should be avoided (maximum of 2 freeze/thaw cycles are allowed). Thawed samples must be mixed gently prior to testing.

9. TEST PROCEDURE

- Bring the kit at temperature room at least 30 minutes before performing.
- Open the pouch and retrieve the devices, sample loop and desicant. Check the color of the desicant. 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 within one hour maximum. Put the device on a flat surface.
- For the first use, tighten the vial cap of clearing buffer provided with the kit in the clockwise direction to pierce the dropper bottle nozzle.

Presentation	Procedure	Indication
A - Sampling		
A.1 - Whole blood sample from finger prick:		
	1. Clean the sampling area (side of the third or the fourth finger) with an antiseptic solution. Allow to dry in the open air.	If the patient is right-handed, choose the 4th finger of his left hand. If the patient is left-handed, choose the 4th finger of his right hand. Do not blow on the finger or dry it with a cloth or paper. After using the disinfectant wipe, put it on his bag. You will use it again to stop the bleeding after taking blood.
	2. Apply the sampling lancet preferably to the side of the finger disinfected (3rd or 4th finger) and press the button on the lancet. Discard the lancet in a sharps container.	
	4. Press your fingertip until a blood drop forms. Take this drop of blood using the sample loop 5µl provided, or with the 5µL laboratory pipette.	The handle should be just full with a thin layer but does not contain too large a drop. Once you have collected a sufficient amount of blood, you can give the alcohol swab to the patient and show him how to use it to stop the bleeding.
	5. With help of the sample loop **of 5 µL provided, or with the 5µL laboratory pipette**, transfer the whole blood drop, from the finger to the **"A" sample well** of the device.	A very simple way to remove the blood in the right place is to push the handle well vertically in the cell until the tip touches the pad. Then, push gently to bend the end of the loop so that the greater part of its surface can reach the membrane.
A.2 - venous blood sample :		
	Use a 5µL laboratory pipette , or use the sample loop of 5µL. And deliver **5 µL of sample** to the **"A" sample well** of the device.	
B – Common procedure :		
	6. Dispense **2 drops** of clearing buffer into **the "B" buffer** well of the device.	Maintain the vial vertically to have well drops.

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67400 ILLKIRCH-GRAFFENSTADEN– France
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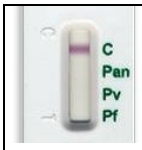
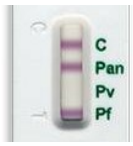
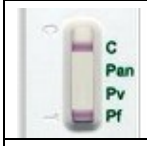
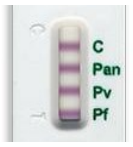
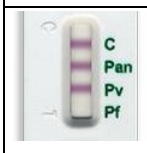
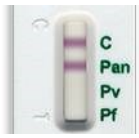
	7. A positive result may appear as early as the first minute. Read results **within 20 minutes. Do not interpret after 30 minutes.** To must be a valid result the band Control C present.	The blood begins to migrate along the membrane surface visible in the results window. The blood will gradually disappear from the results window, leaving only the purple control line and the results (if the patient is positive).

10. RESULTS

	Purple band Control « C » AND No band test T	**The test is negative.** Absence of *Plasmodium* in sample. Do a new test 24 hours after if clinical symptoms persist.
	Purple band Control « C » AND "Pf" purple test band "Pan" purple test band (even if weak intensity)	**The test is positive.** Presence of *Plasmodium falciparum* in sample
	Purple band Control « C » AND "Pf" purple test band (even if weak intensity)	**The test is positive.** Possibility of *falciparum malaria*: post-processing, or early disease (untreated)
	C purple control band AND Pf purple test band Pv purple test band Pan purple test band (even if weak intensity)	**The test is positive.** Presence of Plasmodium falciparum in sample : It is a case of mixed malaria infection as (P.vivax + P.falciparum) or (P.vivax + P.falciparum + Pan) malaria.
	"C" purple control band AND Pv purple test band Pan purple test band (even if weak intensity)	**The test is positive.** Presence of *Plasmodium vivax* in sample
	« C » purple control band AND « Pan »purple band test (even if weak intensity)	**The test is positive.** Presence of *Plasmodium ovale* and/or of *Plasmodium malariae* in sample
	No band control « C »	**The test is invalid:** run the procedure again with a new device. Wrong procedure or high sample viscosity
	No band control « C » AND Test band (Pan and/or Pv and/or Pf)	

11. QUALITY CONTROL

- Internal procedural controls are included in the test. A colored line appearing in the control region (C) is considered an internal positive procedural control, confirming sufficient specimen volume and correct procedural technique.
- External controls are not supplied with this kit. It is recommended to control on each new batch, or on each new delivery. Each laboratory has to set up its own planning of controls. A positive control is available to BIOSYNEX (ref 6120001).

12. TEST LIMITATIONS

- For the reliability of results, respect precautions strictly and follow the procedure. Refer to the results interpretation of instructions for use.
- PALUTOP® + 4 OPTIMA is a diagnostic aid: The test results must be interpreted in the epidemiological, clinical and therapeutic context and must be confirmed by a microscopic reference methods: thick and smear.
- 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. PALUTOP®+4 OPTIMA uses Heterophilic Blocking Reagent to inhibit majority of these interferences.
- In case of infection due to *P. vivax*, to *P. falciparum* or to both, do not forget that the Pan test band will also be positive. Differentiation of infection due to *P. ovale* or to *P. malariae* cannot be done.
- In *P. falciparum* malaria infection, HRP-2 is not secreted in gametogony stage. Hence, in "Carriers", the HRP-2 band may be absent.
- The "Pan" band can be used to monitor success of therapy, in *P. falciparum* malaria cases. Antigen HRP-II (Pf band) can persist during about 15 days after treatment, whereas the pLDH (Pan band) common to 4 species disappears in approximately 5 days. In a few cases, where the Pf band (HRP-2) 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. After 5 to 10 days, if the test reaction is still intensively positive, consider a resistant infection.
- The test can be also used to establish a diagnosis to subjects receiving an antipaludian treatment without any biological diagnosis made previously,
- Usually, the "Pv" and "Pan" bands turn negatives 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.
- In certain *P. ovale* infections, consider an absence of Pan test bands apparition.
- When the parasitemy is lower than the test cut-off, notice that false negative results could appear.
- A false-positive P. vivax band due to a cross-reaction may appear in case of infection with another Plasmodium species, especially if the antigenemia in pLDH is high.[13]

13. PERFORMANCES

P.f. and P.v. Limits of Detection:
The limit of detection for Pf is 100 ppm (parasites per µL) at Pan and Pf bandsThe limit of detection for Pv is 200 ppm (parasites per µL) at Pan and Pv bands.

Sensitivity and specificity of PALUTOP®+4 OPTIMA (in comparison to microscopic method)

Plasmodium species	Number of samples	PALUTOP®+4 OPTIMA		Sensitivity (%)	Specificity (%)
		Positive	Negative		
P. falciparum	16	16	0	**100**	-
P. vivax	25	25	0	**100**	-
No malaria	210	0	210	-	**100**

14. Literature

1) Howard RJ. & al, 1986, *Secretion of a Malarial Histidein-rich Protein (Pf HRP II) from Plasmodium falciparum-infected Erythrocytes*. J. cell Biol 103,1269-1277.
2) Rock EP. & al, 1987, *Comparative Analysis of the plasmodium falciparum Histidine-Rich Proteins HPR-I HPR-II and HPR-III in Malaria Parasites of Diverse Origin*. Parasitol, 95,209-227.
3) Parra ME. & al, 1991, *Identification of Plasmodium falciparum Histidine-Rich Protein 2 in the Plasma of Humans with Malaria*. J. Clin. Microbial. 29,1629-1634
4) Rodriguez-Del Valle M. & al, 1991, *Detection of Antigens and Antibodies in the Urine of Humans with Plasmodium falciparum Malaria*. J. Clin Microbial, 29 1236-1242.
5) Makler MT. & al, 1993, *Parasite lactate assay as an assay for Plasmodium falciparum drug sensitivity*. Am. J. Trop. Med. Hyg. 48(6), 739-741.
6) Piper RC. & 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. & 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. & 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 SM. & al, 1998, *Evaluation of OptiMAL, dipstick test for the diagnosis of malaria*. Ann. Trop. Med. Parasitol., 92, 621-622.
10) Quintana M. & 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 CJ., 1998, *Evaluation of OptiMAL, test for rapid diagnosis of Plasmodium vivax and Plasmodium falciparum*. J. Clin Microbiol. 36(1) 203-206.
12) Moody A. & al, 2000, *Performance of the OptiMAL® malaria antigen capture dipstick for malaria diagnosis and treatment monitoring.* British Journal of Hematology, 109, 1-5.
13) Van Dijk et al, 2009, Evaluation of the Palutop+4 malaria rapid diagnostic test in a non-endemic setting. Malaria Journal 2009, 8:293.

SYMBOLS

	Attention, see instructions for use	LOT	Lot number
IVD	For *in vitro* diagnostic use only		Manufacturer
	Store between 4°C to 30°C		Do not reuse
Σ	Tests per kit	REF	Catalog number
	Expiry	DIL	dilution buffer

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