INSTRUCTION FOR USE
Malaria Test
For Malaria P.f / Pan
Detection in Whole Blood

in vitro diagnostic test

Only for professional *in vitro* diagnostic use

Product Code: TML01

Malaria P.f/Pan Test

BACKGROUND INFORMATION

Malaria is caused by a protozoan which invades human red blood cells. World Health Organization estimates that 3.3 billion were at risks of acquiring malaria in 2006, with 247 million of these developing clinical malaria (86% in Africa), and nearly 1 million (mostly African children) dying from the disease. Microscopic analysis of appropriately stained thick and thin blood smears has been the standard diagnostic technique for identifying malaria infections for more than a century. The technique is capable of accurate and reliable diagnosis when performed by skilled microscopists using defined protocols. The skill of the microscopist and use of proven and defined procedures, frequently present the greatest obstacles to fully achieving the potential accuracy of microscopic diagnosis. Although there is a logistical burden associated with performing a time-intensive, labor-intensive, and equipment-intensive procedure such as diagnostic microscopy, it is the training required to establish and sustain competent performance of microscopy that poses the greatest difficulty in employing this diagnostic technology. The Malaria P.f/Pan Rapid Test Device is a rapid test to qualitatively detect the presence of the *P. falciparum* - specific HRP-II antigens and/or Pan-malarial Aldolase antigens found in P. *falciparum (P.f), P. vivax (P.v), P. ovale (P.o)* and *P. malariae (P.m)*. The test utilizes colloid gold conjugate to selectively detect *P.f*-specific and Pan-malarial antigens (*P.f, P.v, P.o* and *P.m*) in whole blood.

INTENDED USE

Malaria P.f/Pan Test is a rapid chromatographic immunoassay for the qualitative detection of circulating antigens of *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* in whole blood.

REAGENTS

Anti-HRP-II antibodies, anti-Aldolase antibodies and Anti-HRP-II antibodies and anti-Aldolase antibodies conjugated with colloidal gold particles.

METHOD

Malaria P.f/Pan Test uses solid-phase immunochromatographic technology for the qualitative detection of HRP-II antigens and/or Pan-malarial aldolase antigens in human whole blood. Anti-HRP-II antibodies and anti-Aldolase antibodies were immobilized on the P.f and Pan test areas. Anti-HRP-II antibodies and anti-Aldolase antibodies were conjugated with colloidal gold were dried on a conjugate pad. Sample is introduced from sampling pad. If there is HRP-II antigen in the sample, HRP-II antigen binds to the mobile recombinant anti-HRP-II antibodies conjugated with colloidal gold particles. Together they move to the P.f test area. HRP-II antigen – anti-HRP-II antibody conjugated with colloidal gold particles complex binds to the immobilized anti-HRP-II antibodies and as a result of this, HRP-II antigen that have already bound to mobile anti-HRP-II antibodies (conjugated with colloidal gold particles) become immobilized in the P.f test area thus creating a visible colored signal due to the accumulation of colloidal gold particles in the P.f test area (a colored test line), indicating positive test result. If there is Pan-malarial Aldolase antigen in the sample, Pan-malarial Aldolase antigen binds to the mobile recombinant anti-aldolase antibodies conjugated with colloidal gold particles. Together they move to the Pan test area. Pan-malarial Aldolase antigen – anti-aldolase antibody conjugated with colloidal gold particles complex binds to the immobilized anti-aldolase antibodies and as a result of this, Pan-malarial Aldolase antigen that have already bound to mobile anti-aldolase antibodies (conjugated with colloidal gold particles) become immobilized in the Pan test area thus creating a visible colored signal due to the accumulation of colloidal gold particles in the Pan test area (a colored test line), indicating positive test result. If there is no antigen in the sample then sample moves to the P.f and Pan test areas together with unbound (free) anti-HRP-II antibodies and anti-aldolase antibodies conjugated with colloidal gold particles.
Immobilized anti-HRP-II antibodies and/or anti-aldolase antibodies cannot bind to mobilized anti-HRP-II antibodies and anti-aldolase antibodies conjugated with colloidal gold particles, therefore no visible colored signal in P.f and Pan test areas (no colored test line) can be obtained, indicating negative test result. Regardless of HRP-II antigen and Pan-malarial Aldolase antigen content of the liquid sample, accumulation of colloidal gold particles produces a visible colored signal in the control area "C" (a colored control line), indicating a valid test result. Colored line should be visible in the control area "C" in every case; if no visible colored line in control area "C", test result should be indicated as invalid.

PRECAUTIONS AND LIMITATIONS

1. For Professional and *in vitro* diagnostic use only.
2. Do not use test kit beyond expiry date. The test device is single use. Do not reuse.
3. The test device should remain in its original sealed pouch until usage. Do not use the test if the seal is broken or the pouch is damaged.
4. Wear disposable gloves while performing the test.
5. Use a new dropper for each sample. Plastic dropper supplied with test kit may not drop exact sample volume thus micropipette should be used.
6. All patient samples should be handled as taking capable of transmitting disease into consideration. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of samples.
7. This test will indicate only the presence or absence of *Plasmodium sp.* (*P.f, P.v, P.o, P.m*) in the sample, and should not be used as the only basis for the diagnosis of malaria infection. As with all diagnostic tests, it should be kept in mind that an identification diagnosis can't be based on a single test result. Diagnosis can only be reached by an expert after the evaluation of all clinical and laboratory findings.

STORAGE

Test device should be kept away from direct sunlight, moisture, heat and radiation sources. Store at 4 - 30°C (39 - 86°F). Do not freeze.
The test in the original packaging retains stable until expiry date at storage conditions. The test device should be used in maximum one hour after the foil is opened.

Kit components: Test cassettes, droppers, diluent and instructions for use.
Additional materials required but not provided: Sample collection tube, centrifuge and timer, lancet (for only fingerstick whole blood), heparinized dispensing bulbs and capillary tubes (for only fingerstick whole blood).
Additional materials recommended but not provided: Micropipettes to deliver mentioned amount of sample in the test procedure, negative and positive control materials

SAMPLE COLLECTION AND PREPARATION

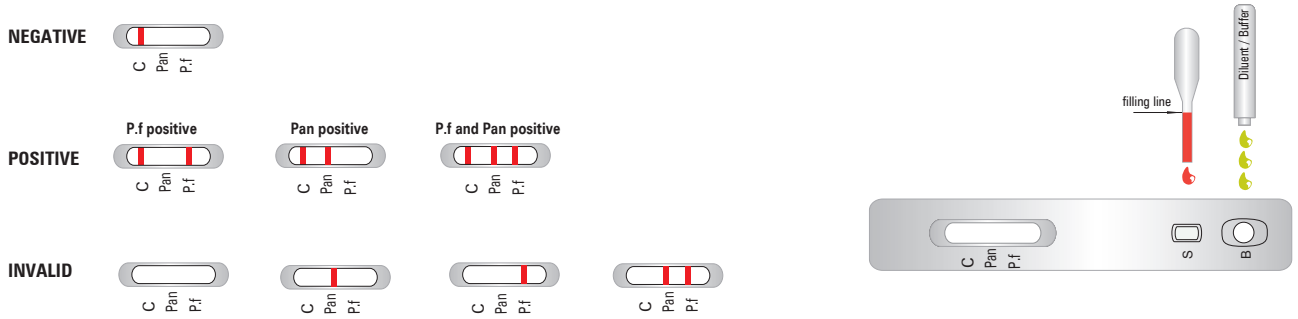
The test can be performed using whole blood. Test should be performed immediately with whole blood samples. Otherwise, whole blood samples should be stored at 2 - 8 °C with anticoagulants (EDTA, heparin, citrate should be used) to avoid coagulation until they are being tested in a period of 2 days after collection. Do not freeze whole blood sample.

TEST PROCEDURE

1. Bring the tests and whole blood samples to room temperature. Take the test out of its pouch.
2. Draw whole blood into fill line of dropper as shown illustration below. Put 1 drop (5 μl) into the sample well (S) of the cassette. Immediately after, 3 drops of diluent is added into the buffer well (B) and allowed to soak in.
   Avoid the formation of any air bubbles.
3. Depending on the HRP-II or Aldolase antigen concentration in the sample, the test can react even in 5 minutes. Results should be read at 10 minutes as shown below. Do not interpret results beyond 20 minutes, results forming after 20 minutes should be regarded as invalid.

INTERPRETATION OF RESULTS

Negative : Only one colored line appears in the "C" control area.
Positive: * Two or Three distinct colored lines appear.
***P. falciparum* or mixed malaria infection:** One line appears in the "C" control area, one line appears in Pan test area and one line appears in P.f test area.
***P. falciparum* infection:** One line appears in the "C" control area, and one line appears in P.f test area.
Non-*falciparum Plasmodium species* infection: One line appears in the "C" control area and one line appears in Pan test area.
The color intensity of P.f or Pan test lines may vary depending on the concentration of antigens HRP-II or Aldolase present in the specimen.
Invalid: No colored line is visible in "C" area or one colored line is visible in P.f test area or one colored line is visible in Pan test area. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device.



QUALITY CONTROL

Tests have built in procedural quality control features. When the test is complete, the user will see a colored line in the "C" area of the test on negative samples and colored lines in the P.f and/or Pan Test areas and "C" area on positive samples. The appearance of the control "C" line is considered as an internal procedural control. This line indicates that sufficient volume of sample was added as well as valid test result. It is recommended that a negative control and a positive control be used to verify proper test performance as an external control. Users should follow appropriate federal, state and local guidelines concerning the external quality controls.

PERFORMANCE EVALUATION

Malaria P.f/ Pan Test has been tested with thin or thick microscopy on clinical samples. The results are as below

For Pan:
Sensitivity : 99,9 % Specificity : 99,9 % + Predictive V : 99,9 % - Predictive V : 99,9 %

For P.f:
Sensitivity : 99,9 % Specificity : 99,1 % + Predictive V : 94,6 % - Predictive V : 99,9 %

Method			Microscopy			Total Results
	Results		Positive		Negative	
			Pan	P.f		
Malaria P.f/Pan Test	Positive	Pan	158	0	0	158
		P.f	0	53	3	56
	Negative		0	0	324	324
Total Results			211		327	538

Note: The comparison for Pan line has been only done with blood specimens positive with *Plasmodium vivax* specimen. The claims for Pan lines are based on scientific findings that Pan-malarial Aldolase is found in other malarial parasites including *Plasmodium ovale* and *Plasmodium malariae.*

Intra-Assay
The run precision has been determined by using 10 replicates of specimens containing negative, low and high positive samples. The negative and positive values were correctly identified.
Inter-Assay
Between run precision has been determined by using the same specimens of negative, low positive and high positive of 10 independent assays and with Malaria P.f/pan Test. The negative and positive values were correctly identified.

REFERENCES

1. Bill MaConell, Malaria Laboratory Diagnosis. January 2001.
2. WHO, WHO World Malaria Report 2008. 2008, WHO – Global Malaria Programme: Geneva
3. Cooke AH, Chiodini PL, Doherty T, et al, Comparison of a parasite lactate dehydrogenase-base immunochromatographic antigen detection assay with microscopy for the detection of malaria parasite in human blood samples. Am J Trop Med Hyp,1999, Feb: 60(2):173-2.

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CE
Manufacturer
Consult instruction for use
Attention, see instruction for use
IVD In vitro diagnostic medical device
For single use only
Number of test
REF Catalog number
Storage temperature
LOT Lot number
Expiry date