Malaria Pf/Pan Antigen Rapid Test Cassette (Colloidal Gold)

Cassette

Instruction for Use

A lateral flow immunochromatographic assay for the qualitative simultaneous detection and differentiation of HRP-2 (Histidin- rich protein-2) specific to Plasmodium falciparum (Pf) and pLDH (Plasmodium lactate dehydrogenase) specific to Plasmodium species (Pan) in human whole blood.

CE IVD REF

In Vitro Diagnosis
For Professional Use

PROUDCT SPECIFICATION

1T/BOX,10T/BOX,20T/BOX,25T/BOX,40T/BOX，50T/BOX

INTENDED USE

Malaria Pf/Pan Antigen Rapid Test Cassette (Colloidal Gold) is a lateral flow immunochromatographic assay for the qualitative simultaneous detection and differentiation of HRP-2 (Histidin- rich protein-2) specific to Plasmodium falciparum (Pf) and pLDH (Plasmodium lactate dehydrogenase) specific to Plasmodium species (Pan) in human whole blood specimen as an aid in the diagnosis of Malaria infection. The test is recommended for professional In Vitro Diagnostic use only. All results must be interpreted together with other clinical information available to the physicians.

SUMMARY

Malaria is a serious parasitic disease characterized by fever, chills, and anemia and is caused by a parasite that is transmitted from one human to another by the bite of infected Anopheles mosquitoes. There are five species of Plasmodium can infect and be transmitted by humans. Severe disease is largely caused by Plasmodium falciparum while the disease caused by Plasmodium vivax, Plasmodium ovale[1], and Plasmodium malariae is generally a milder disease that is rarely fatal. Plasmodium knowlesi is a zoonosis that causes malaria in macaques but can also infect humans.[2,3] In humans, the parasites (called sporozoites) migrate to the liver where they mature and release another form, the merozoites. The disease now occurs in more than 90 countries worldwide, and it is estimated that there are over 225 million clinical cases and 78,1000 malaria-caused deaths per year.[4]

At the present, malaria is diagnosed by looking for the parasites in a drop of blood. Blood will be put onto a microscope slide and stained so that the parasites will be visible under a microscope.

Malaria Pf/Pan Antigen Rapid Test Cassette (Colloidal Gold) detects all the four kinds of malaria by detecting Malaria P.falciparum specific histidine rich protein-2 (Pf HRP-2) (for Pf) and the pLDH (for Pan, the all four types of malaria) released during plasmodium infection. It can be performed easily without extra laboratory equipment and it can also be used to monitor the treatment of anti-malaria since that the pLDH is undetectable 2 weeks after the elimination of the protozoa.

PRINCIPLE

Malaria Pf/Pan Antigen Rapid Test Cassette (Colloidal Gold) is a lateral flow immunochromatographic assay. Malaria is caused by a protozoan which invades human red blood cells. Malaria is one of the world's most prevalent diseases. According to the WHO, the worldwide prevalence of the disease is estimated to be 300-500 million cases and over 1 million deaths each year. Most of these victims are infants, young children. Over half of the world's population lives in malarious areas. 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 Pf/Pan Antigen Rapid Test Cassette is a rapid test to qualitatively detect the presence of P. falciparum - specific HRP-II and four kinds of circulating plasmodium falciparum(P. falciparum (P.f.), P. vivax (P.v.), P. ovale (P.o.), and P. malariae (P.m.)). The test utilizes colloidal gold conjugate to selectively detect P.f-specific and Pan-malarial antigens (P.f., P.v., P.o. and P.m.) in whole blood. The membrane is pre-coated with anti-HRP-II antibodies and anti-Aldolase antibodies. During testing, the whole blood specimen reacts with the dye conjugate, which has been pre-coated on the test cassette. The mixture then migrates upward on the membrane by capillary action, reacts with anti-Histidine-Rich Protein II (HRP-II) antibodies on the membrane on P.f. Test Line region and with anti-Aldolase antibodies on the membrane on Pan Line region. If the specimen contains HRP-II or Plasmodium-specific Aldolase or both, a colored line will appear in P.f. line region or Pan line region or two colored lines will appear in P.f. line region and Pan line region. The absence of the colored lines in P.f. line region or Pan line region indicates that the specimen does not contain HRP-II and/or Plasmodium-specific Aldolase. To serve as a procedure control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

PRECAUTIONS

1. For professional and IN VITRO diagnostic use only.
2. The test should remain in the sealed pouch until use.
3. Do not use the kit if the foil pouch is punctured or not well sealed.
4. Do not reuse or use kits after the expiration date.
5. Do not mix components from kits with different lot number.
6. Avoid microbial contamination of reagents.
7. Wear gloves during the whole process and avoid reagents or specimen spilling-out. Wash hands thoroughly afterwards.
8. Dispose the used kit after decontamination of all liquids or solid wastes by following the local law or laboratory rule.

STORAGE & STABILITY

Store the kit in cool and dry places at a temperature between 2-30°C. **Do not freeze.** The shelf-life of the kit under these storage conditions is 24 months.

MATERIALS

Materials Provided:

Test Devices, each test cassette is packed in a foil pouch with a dropper and a package of desiccant.
Assay Buffer
Instructions for Use

Materials May Need But Not Provided:

Lancet
Timer
Alcohol pad

SPECIMEN COLLECTION & PREPARATION

Whole blood specimen is used for Malaria Pf/Pan Antigen Rapid Test Cassette (Colloidal Gold). Drops of whole blood can be obtained by either finger tip puncture or venipuncture. Do not use any hemolyzed blood for testing.

To collect Fingerstick Whole Blood specimens using a lancet:

1) Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
2) Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
3) Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
4) Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.

Add the Fingerstick Whole Blood specimen to the specimen well of the test device by using the specimen dropper provided:

Touch the open end of the dropper to the blood until filled to approximately 5μl (see below the illustration of 5μl mark line). Avoid air bubbles. Squeeze the dropper bulb gently to dispense the whole blood to the specimen well [S] of the test cassette.

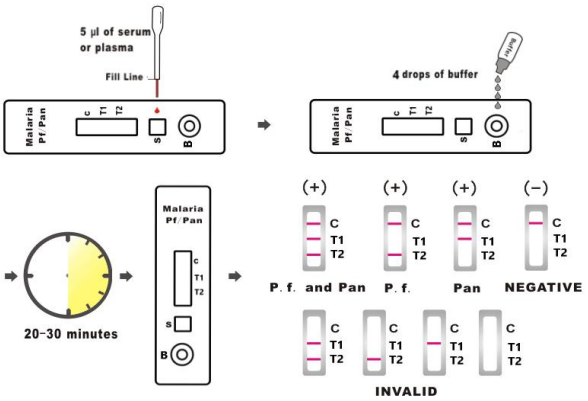
Collection by venipuncture

1) Collect whole blood into a collection tube (containing EDTA, citrate or heparin) by venipuncture.
2) If specimens are not immediately tested, they should be refrigerated at 2 ~ 8°C. For storage periods greater than three days, freezing is recommended. They should be brought to room temperature prior to use. Using the specimen after long-term storage of more than three days can cause non-specific reaction.
3) When stored at 2 ~ 8°C, the whole blood sample should be used within three days.

ASSAY PROCEDURES

Allow test device, buffer, specimen, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.

1) Remove the test cassette from the foil pouch and place it on a clean and level surface. Be sure to label the device with specimen's ID number.
2) Use the dropper provided to draw whole blood specimen just over the Specimen Line as shown below and add 5 μl of whole blood into sample well [S]. (Please refer to illustration)
   *A 5 μl volume pipette can be used instead of the provided dropper for a better precision.
3) Add 4 drops (about 160-200μl) of lysis buffer into buffer well [B] (Please refer to illustration). **For high viscous blood, if migration (the wetting of membrane) is not observed in the test window after 5 minutes, add additional 1 drop of buffer.**
4) Start timer and read results in 20-30 minutes. **Do not read results after 30 minutes.**



INTERPRETATION OF RESULTS

(Please refer to the illustration)

NEGATIVE: Only one colored line appears on the control (C) region. No apparent line on the test region (line of Pf and Pan).

POSITIVE:

Three distinct red lines appear in the control region (C), the T1 test region (T1) and the T2 test region (T2), indicating the P. falciparum, and P. vivax or P. ovale or P. malariae or mixed infection.

Two distinct red lines appear. One line should be in the control region (C) and another line should be in the T1 test region (T1), indicating the P. vivax or P. ovale or P. malariae or mixed infection.

Two distinct red lines appear. One line should be in the control region (C) and another line should be in the T2 test region (T2), indicating the P. falciparum infection.

INVALID:

No red bands appear or control line fails to appear, indicating that the operator error or reagent failure.

PERFORMANCE

Sensitivity and specificity: Comparative testing with the traditional Microscopic examination method shows that Malaria Pf/Pan Antigen Test is capable to detect out malaria infection when in blood specimen plasmodium protozoa of Plasmodium falciparum reaches 50pcs/ul or plasmodium protozoa of the other species P. vivax, P. ovale, and P. malariae reach 50pcs/ul.

Clinical study between Malaria Pf/Pan Antigen Test and Microscopic examination method demonstrates that Malaria Pf/Pan Antigen Test has a sensitivity of 99.37% and specificity of 99.39% for P.falciparum. The test results are shown in below table1:

Table 1:

Malaria P.falciparum Evaluation Result		Microscopic examination method		Total
		Positive	Negative	
Malaria Pf/Pan Ag test	Positive	314	5	319
	Negative	2	810	812
Total		316	815	1131

Relative Sensitivity: 314 /（314 + 2）×100%=99.37%
Relative Specificity: 810 /（5 + 810）×100% =99.39%
Overall Agreement: (314 + 810)/（314+ 2 + 5 + 810）×100%=99.38%

Clinical study between Malaria Pf/Pan Antigen Test and Microscopic examination method demonstrates that Malaria Pf/Pan Antigen Test has a sensitivity of 99.0%, and specificity of 99.53% for P. vivax. The test results are shown in below table2:

Table 2:

Malaria P. vivax Evaluation Result		Microscopic examination method		Total
		Positive	Negative	
Malaria Pf/Pan Ag test	Positive	281	4	285
	Negative	3	843	847
Total		284	847	1131

Relative Sensitivity: 281 /（281+3）×100%=99.0%
Relative Specificity: 843 /（4+ 843）×100% =99.53%
Overall Agreement: (281 + 843)/（281 + 3 + 4 + 843）×100%=99.38%

Precision: Within-run and between-run have been determined by the testing 10 replicates of four specimens: a negative, a low positive, a medium positive and a strong positive. All values were correctly identified 100% of the time.

Interference: Clinical evaluation results of Malaria P.f/Pan Antigen Rapid Test (Colloidal Gold Method) show almost no interference with commonly diseases which may cause similar febrile symptoms.

LIMITATIONS

1. The Malaria P.f/Pan Antigen Rapid Test (Colloidal Gold Method) is for in vitro diagnostic use only. This test should be used for the detection of P.f, P.v, P.o, P.m antigens in whole blood specimens only. Neither the quantitative value nor the rate of increase in P.f, P.v, P.o, and P.m concentration can be determined by this qualitative test.
2. The Malaria P.f/Pan Antigen Rapid Test (Colloidal Gold Method) will only indicate the presence of antigens of Plasmodium sp. (P.f, P.v, P.o, P.m) in the specimen and should not be used as the sole criterion for the diagnosis of malaria infection.
3. As known relevant interference, haemolytic samples, rheumatoid factors-contained samples and lipaemic, icteric samples can lead to impair the test results.
4. In a few cases, where the Pf band is positive and the Pan 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.
5. The test is limited to the detection of antigen to Malaria Plasmodium sp. Although the test is very accurate in detecting HRP-II specific to P. falciparum or pLDH specific to plasmodium species (P. falciparum, vivax, malariae, ovale), a low incidence of false results can occur. Other clinically available tests are required if questionable results are obtained.
6. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of malaria infection.
7. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

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2. Fong YL, Cadigan FC, Coatney GR (1971). "A presumptive case of naturally occurring Plasmodium knowlesi malaria in man in Malaysia". Trans. R. Soc. Trop. Med. Hyg. 65 (6): 839–40
3. Singh B, Kim Sung L, Matusop A et al. (March 2004). "A large focus of naturally acquired Plasmodium knowlesi infections in human beings". Lancet 363 (9414): 1017–24
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Index of Symbols

IVD	For in vitro diagnostic use only		Do not reuse
	Expiry date		See instruction for use
	Warning, please refer to the instructions in the annex		Manufacturer
2℃ 30℃	Temperature scope within which the product is reserved	LOT	Batch number
REF	Catalog #	Σ	Tests / box
EU REP	European union authorized representative		Keep dry
	Keep away from sunlight		Don't use the product when the package is damaged
	Biological risks		
CE	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC		

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