Rapid Response™

Malaria P.f./P.v. Test Cassette
(Whole Blood)

REF MAV-11C40 Product Insert

A rapid test for the qualitative detection of circulating antigens of P. falciparum (P.f.) and P vivax (P.v.) in whole blood.

For professional *in vitro* diagnostic use only.

Intended Use

The Rapid Response™ Malaria P.f./P.v. Test Cassette is a rapid chromatographic immunoassay for the qualitative detection of two kinds of circulating plasmodium falciparum (P. falciparum (P.f.) and P. vivax (P.v.)) in whole blood.

Introduction

Malaria is caused by a protozoan which invades human red blood cells.[1] 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.[2] 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 Rapid Response™ Malaria P.f./P.v. Test Cassette is a rapid test to qualitatively detect the presence of P. falciparum -specific HRP-II and P. vivax (P.v.). The test utilizes colloid gold conjugate to selectively detect P.f-specific and P. vivax (P.v.)-specific antigens in whole blood.

Principle

The Rapid Response™ Malaria P.f./P.v. Test Cassette is a qualitative, membrane-based immunoassay for the detection of P.f. and P.v. antigens in whole blood. The membrane is pre-coated with anti-HRP-II antibodies and anti-pLDH 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-pLDH antibodies on the membrane on P.v. Line region. If the specimen contains HRP-II or Plasmodium-specific P. vivax LDH or both, a colored line will appear in P.f. line region or P.v. line region or two colored lines will appear in P.f. line region and P.v. line region. The absence of the colored lines in P.f. line region or P.v. line region indicates that the specimen does not contain HRP-II and/or Plasmodium-specific P. vivax LDH. 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.

Reagents

The test cassette contains anti-HRP-II of Plasmodium falciparum antibodies conjugated gold and anti-Plasmodium falciparum P.vivax LDH antibodies conjugated gold and anti-HRP-II antibodies and anti-pLDH antibodies coated on the membrane.

Precautions

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- For whole blood specimen use only. Do not use other specimens.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- The used test should be discarded according to local regulations.
- Humidity and temperature can adversely affect results.
- Do not exchange or mix buffer and test cassettes from kits of different lot numbers.
- Caution must be taken at the time of specimen collection. Inadequate volume of specimen may lead to lower sensitivity.
- Be sure to add sufficient buffer to the cassette's sample well. Invalid result may occur if inadequate buffer is added.

Materials

Materials provided

- Individually packed test cassettes
- Disposable specimen droppers
- Buffer
- Product insert

Materials required but not provided

- Specimen collection container
- Pipette and disposable tips
- Timer
- Lancets

Storage and Stability

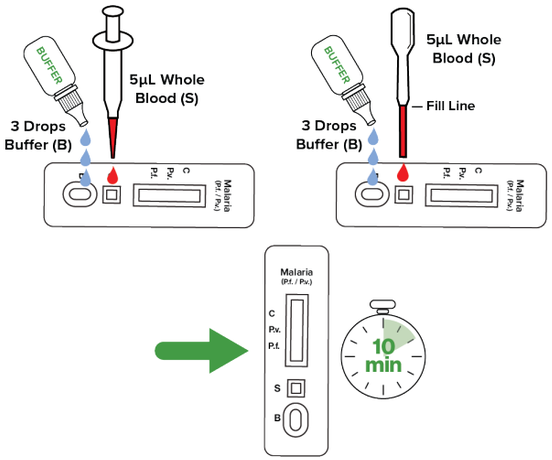
The kit can be stored at room temperature or refrigerated (35.6-86°F; 2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

Collection and Storage of Specimens

- The Rapid Response™ Malaria P.f./P.v. Test Cassette can be performed using whole blood.
- Both fingerstick whole blood and venipuncture whole blood can be used.
- To collect fingerstick whole blood specimens:
- Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
- Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
- Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
- Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
- Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Whole blood collected by venipuncture should be stored at 35.6-46.4°F (2-8°C) if the test is to be run within 2 days of collection. For long term storage, specimens should be kept below -4°F (-20°C). Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly for more than three times.
- If specimens are to be shipped, they should be packed in compliance with federal regulations covering the transportation of etiologic agents.

Test Procedure

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible.
2. Place the cassette on a clean and level surface.
   For Whole Blood specimen:
   - Use a pipette: To transfer 5µL of whole blood to the specimen well, then add 3 drops of buffer (approximately 180µL).
   - Use a disposal specimen dropper: Hold the dropper vertically, draw the specimen up to the Fill Line as shown in illustration below (approximately 5µL). Transfer the specimen to the specimen well, then add 3 drops of buffer (approximately 180µL), and start the timer.
3. Wait for the colored line(s) to appear. Read results at **10 minutes**. Do not interpret the result after 20 minutes.

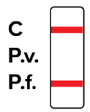


Results Interpretation

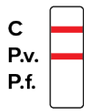
POSITIVE:*

Two or Three colored lines appear.

***P. falciparum* or mixed malaria infection:** one line appears in the control region, one line appears in P.v. line region and one line appears in P.f. line region.



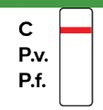
***P.falciparum* infection:** one line appears in the control region, and one line appears in P.f .line region.



Non-falciparum Plasmodium species infection: one line appears in the control region and one line appears in P.v. line region.

***NOTE:** The color intensity of P.f. or P.v. test lines may vary depending on the concentration of antigens, viz., HRP-II or P. vivax LDH present in the specimen.

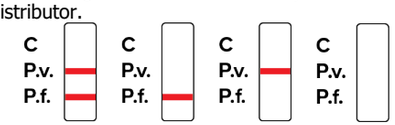
NEGATIVE:



Only one colored line appears in the control region.

INVALID:

Control line fails to appear. 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. If the problem persists, discontinue using the test kit immediately and contact your local distributor.



Quality Control

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is an internal procedural control. It confirms sufficient specimen volume and correct procedural technique.

Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

Limitations

1. The Rapid Response™ Malaria P.f./P.v. Test Cassette is for *in vitro* diagnostic use only. This test should be used for the detection of P.f. and P.v antigens in whole blood specimens only. Neither the quantitative value nor the rate of increase in P.f. and P.v., concentration can be determined by this qualitative test.
2. The Rapid Response™ Malaria P.f./P.v. Test Cassette will only indicate the presence of antigens of Plasmodium sp. (P.f. and P.v.) in the specimen and should not be used as the sole criterion for the diagnosis of malaria infection.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. 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.

Expected Values

The Rapid Response™ Malaria P.f./P.v. Test Cassette has been compared with traditional thick and thin blood films microscopic analysis. The correlation between the two systems is over 99.0%.

Performance Characteristics

Sensitivity

The Rapid Response™ Malaria P.f./P.v. Test Cassette has been tested with microscopy on clinical samples. The results show that the sensitivity of the Rapid Response™ Malaria P.f./P.v. Test Cassette is >99.9% when compared to results obtained with microscopy.

Specificity

The Rapid Response™ Malaria P.f./P.v. Test Cassette uses antibodies that are highly specific to Malaria P.f.-specific and P. vivax LDH antigens in whole blood. The results show that the specificity of the Rapid Response™ Malaria P.f./P.v. Test Cassette is >99.9%, when compared to results obtained with microscopy.

Method		Microscopy			Total Results
Rapid Response™ Malaria P.f./P.v. Test Cassette	Results	Positive		Negative	
		P.v.	P.f.		
	Positive	54*	85**	0	134
	Negative	1	0	500	501
Total Results		55	85	500	635

Comment: Blood Samples infected by Plasmodium falciparum (n = 85), Plasmodium vivax (n = 54) were included, as well as 500 malaria negative samples to be confirmed with microscopy.
Note: *There was one P. vivax sample to show a P.v. line and a P.f. line.
** There were two P. falciparum samples that they both showed a P.v. line and a P.f. line.
Relative Sensitivity for P.f.-specific antigens: 85/85>99.9 %(95%CI***: 96.5%~100.0%)
Relative Sensitivity for P.v. antigens: 54/55=98.2% (95%CI***: 90.3%~100.0%)
Relative Specificity: 500/500>99.9% (95%CI***: 99.4%-100.0%)
Accuracy: (54+85+500)/(54+85+1+500)=99.8% (95%CI***: 99.1%-100.0%)
*** Confidence Intervals

Minimum Detection Level

Type	Parasites/µL
P.falciparum	200
P. vivax	1500

Precision Intra-Assay

Within-run precision has been determined by using 15 replicates of four specimens: a negative, a P.f. positive, a P.v. positive and an P.f./P.v. dual positive. The specimens were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 15 independent assays on the same four specimens: negative, a P.f. positive, a P.v. positive and an P.f./P.v. dual positive. Three different lots of the Rapid Response™ Malaria P.f./P.v. Test Cassette have been tested using these specimens. The specimens were correctly identified >99% of the time.

Cross-reactivity

The Rapid Response™ Malaria P.f./P.v. Test Cassette (Whole Blood) has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, Syphilis, HIV, HCV, H. Pylori, MONO, CMV, Rubella and TOXO positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to Malaria negative and positive specimens.

Acetaminophen: 20 mg/dL	Caffeine: 20 mg/dL
Acetylsalicylic Acid: 20 mg/dL	Gentisic Acid: 20 mg/dL
Ascorbic Acid: 2 g/dL	Albumin: 2 g/dL
Creatin: 200 mg/dL	Bilirubin: 1g/dL
Oxalic Acid: 60 mg/dL	

None of the substances at the concentrations tested interfered in the assay.

Bibliography

1. Bill MaConell, Malaria Laboratory Diagnosis. January 2001.
2. Cooke AH, Chiodini PL, Doherty T, et al, Comparison of a parasite lactate dehydrogenase-baseimmunochromatographic 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.

Glossary of Symbols

Consult instructions for use
Test per Kit
EC REP Authorized Representative
Store between 35.6°F to 86°F
Use by
Do Not Reuse
LOT Lot Number
IVD For *in vitro* diagnostic use only
REF Catalogue #

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