

MALARIA P. FALCIPARUM / P. VIVAX

1. EXPLANATION OF THE TEST:

Malaria is a serious, sometimes fatal, 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 mosquito. There are four kinds of malaria that can infect humans. *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. In humans, the parasites (called sporozoites) migrate to the liver where they mature and release another form, the merozoites. The disease is a major health problem in much of the tropics and subtropics. More than 200 million people in the world have malaria.

At 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. At the most recent, clinical diagnostic issues related to malaria are the detection of malaria antibodies in human blood or serum by immunoassay. The ELISA format and immuno- chromatographic format (rapid) to detect antibody of malaria are available recently.

Malaria P.f / P.v rapid test is an immuno- chromatographic (rapid) test for the qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) specific to *Plasmodium falciparum* and *Plasmodium vivax* simultaneously in human serum, plasma or whole blood.

Malaria P.f / P.v rapid test contains a membrane strip, which is pre-coated with recombinant malaria. P.f capture antigen (MSP, CSP) on test band 1 region and with recombinant malaria. P.v capture antigen (MSP, CSP) on test band 2 region. The recombinant malaria P.f / P.v antigen (MSP, CSP) -colloid gold conjugate and serum sample moves along the membrane chromatographically to the test region (1,2) and forms a visible line as the antigen antibody-antigen gold particle complex forms with high degree of sensitivity and specificity. This test device has a letter of 1, 2 and C as "Test Line 1" "Test Line 2" and "Control Line" on the surface of the case. Both the Test Lines and Control Line in result window are not visible before applying any samples. The Control Line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working.

2. MATERILAS PROVIDED:

Malaria P.f / P.v test kit contains following items to perform the assay.

- 1) Malaria P.f / P.v test device.
- 2) Assay Diluents
- 3) Instructions for use

3. PRECAUTIONS:

Malaria P.f / P. v test devices should be stored at room temperature. The test device is sensitive to humidity and as well as to heat. Perform the test immediately after removing the test device from the foil pouch. Do not use it beyond the expiration.

4. SPECIMEN COLLECTION AND STORAGE:

- a) Collect the whole blood using the suitable anti- coagulant.
- b) Centrifuge whole blood to get plasma / serum specimen.
- c) 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.
- d) Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.
- e) The whole. blood may be used for testing immediately or may be stored at 2-8° C up to three days.

5. WARNINGS:

- a) For *in vitro* diagnostic use only.
- b) Do not eat or smoke while handling specimens.
- c) Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
- d) Avoid splashing or aerosol formation.
- e) Clean up spills thoroughly using an appropriate disinfectant.
- f) Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious waste, in a biohazard container.
- g) Do not use the test kit if the pouch is damaged or the seal is broken.

6. PROCEDURE:

- a} Remove the test device from the foil pouch, and place it on a flat, dry surface.
- b) Slowly add 10ul of serum, plasma or 20ul of whole blood to the sample well (S) and then add 3 drops of the assay diluent (Figure 1).
- c} As the test begins to work, you will see purple color move across the result window in the center of the test device.
- d) Interpret test results at 5 -20 minutes. Do not interpret after 20 minutes.

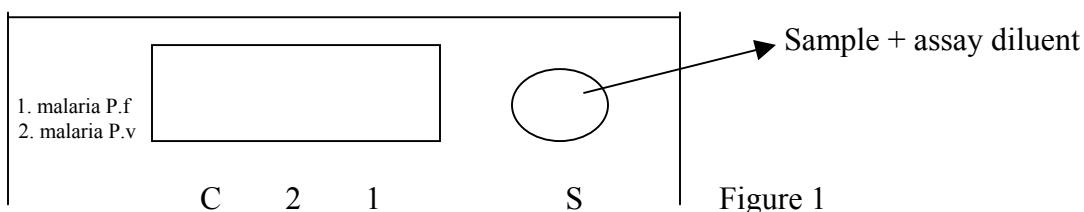


Figure 1

Caution: The above interpreting time is based on reading the test results at room temperature of 15 - 30°C. If your room temperature is significantly lower than 15°C, then the interpreting time should be properly increased.

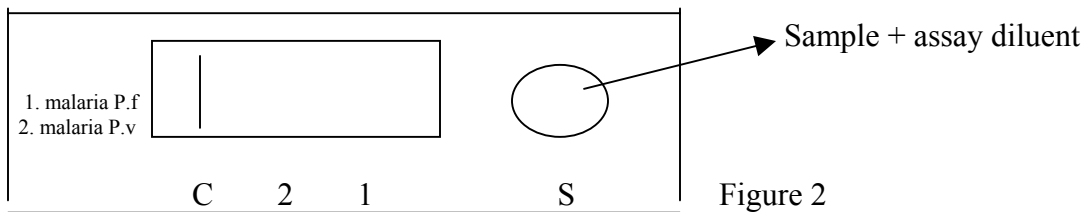
7. INTERPRETATION OF THE TEST:

a) A color band will appear in the left section of the Result Window to show that the test is working properly. This band is the Control Band.

b) The right section of the Result Window indicates the test results. If another color band appears in the right section of the Result Window, this band is the Test Band 1 of P f or / and Test Band 2 of P v

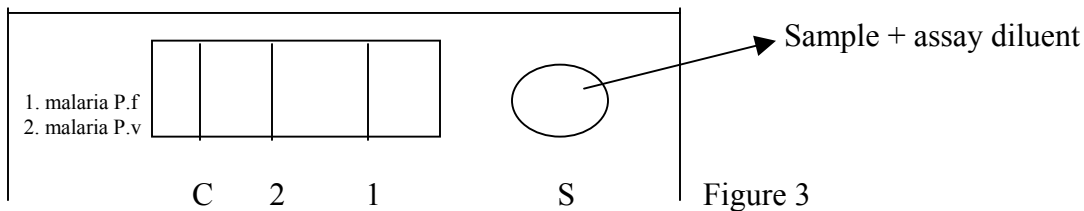
NEGATIVE RESULT: ONE COLOR BAND:

The presence of only one band within the Result Window indicates a negative result (Figure 2).



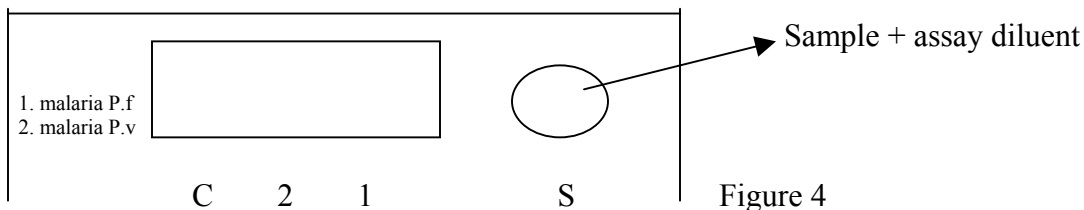
POSITIVE RESULT:

The presence of not less than two color bands ("1" band, "2" band and "C" band) within the Result Window, no matter which band appears first, indicates a positive result (Figure 3) for P.f or /and P.v respectively.



INVALID RESULT:

If the purple color band is not visible within the Result Window after performing the test, the result is considered invalid (Figure 4). The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be re- tested.



8. LIMITATIONS OF THE TEST:

The test is limited to the detection of antibodies to Malaria both *Plasmodium falciparum* and *Plasmodium vivax*. Although the test is very accurate in detecting antibodies to Malaria P.f / P.v, a low incidence of false results can occur. Other clinically available tests are required if questionable results are obtained. 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.

9. PERFORMANCE CHARACTERISTICS:

Sensitivity and Specificity:

Malaria P. f / P .v rapid test have tested with positive and negative clinical samples tested by microscopic examination of whole blood

1) Malaria P.f evaluation results

Reference		Malaria Pf / P v rapid test			
Method	Result	Positive (T1)	Positive (T2)	Negative	Total results
Microscopic examination	P.f positive	150	0	20	170
	P.f Negative	3	0	197	200
Total results		153	0	217	370

In a comparison of Malaria P.f / P.v rapid test versus microscopic examination of whole blood, results gave sensitivity of 88.2% (150/170) a specificity of 98.5% (197/200), and a total agreement of 93.8% (347/370).

(2) Malaria P.v evaluation results

Reference		Malaria Pf / P v rapid test			
Method	Result	Positive (T1)	Positive (T2)	Negative	Total results
Microscopic examination	P.v positive	0	158	15	173
	P.v Negative	0	3	205	208
Total results		0	161	220	381

In a comparison of Malaria P.f / P.v rapid test versus microscopic examination of whole blood, results gave sensitivity of 91.3% (158/173), a specificity of 98.5% (205/208), and a total agreement of 95.3% (363/381).

b) Precision:

(1) Within run, precision was determined by using 10 replicates of four different specimens containing different concentrations of antibody. The negative and positive values were correctly identified 100% of the time.

(2) Between run precision was determined by using the four different specimens containing different concentrations of antibody in three different replicates with three different lots of test devices. Again negative and positive results were observed 100% of the time.

10. BIBLIOGRAPHY OF SUGGESTED READING:

- 1) David R. and et. Al. A Longitudinal Study of Type- Specific Antibody Responses to *Plasmodium falciparum* Merozoite Surface Protein -1 in an Area of Unstable Malaria in Sudan. *Journal of Immunology'* 161 : 347-359 (1998).
- 2) Alon Warburg and Imogene Schneider. In Vitro Culture of the Mosquito Stages of *Plasmodium falciparum*. *Experimental Parasitology* 76, 121- 126 (1993)
- 3) Helen L. Gibson, Jeffrey E. Tucker: Structure and expression of the gene for Pv200, a major blood- stage surface antigen of *Plasmodium vivax*. *Molecular and Biochemical Parasitology*, 50 (1992) 325-334
- 4) Arthur E.Brown, H.Kyle Webster : Characteristics of Natural Antibody Responses to the Circumsporozoite protein of *Plasmodium vivax*. *Am. J. Trop. Med. Hyg.*, 44(1), 1991,p.21-27 (90- 173)

2, Sheetal, 8th Main, 2nd Cross, Byrasandra Layout,
Jayanagar I Block East, Bangalore 560 011. India.
Phone: + 91-80-2654 4588 / 2654 4589 Fax: + 91-80-2245 7314.
Email: tashima@vsnl.com
Web: www.tashima.net
