

Health-Chem Diagnostics, LLC

3341 S.W. 15th Street – Pompano Beach, FL 33069 - USA – Phone: (954) 979-3845 – Fax: (954) 979-7997
Website: www.healthchemdiagnostics.com

ONE STEP™ MALARIA PF/PV CASSETTE TEST - ANTIGEN DETECTION - (WHOLE BLOOD)

Explanation of the Test

The One Step Malaria (p.f/p.v) Cassette Test is a two site sandwich immunoassay utilizing whole blood for the detection of *P. falciparum* specific histidine rich protein-2 (Pf. HRP-2) and *P. vivax* specific pLDH. The test can also be used for specific detection and differentiation of *P. falciparum* and *P. vivax* malaria.

Principle of the Procedure

A capture monoclonal antibody is immobilized on the membrane within the cassette. The red blood cells are lysed releasing Pf. HRP-11 and *P. vivax* specific pLDH which binds selectively to this antibody as the blood flows along the strip inside the cassette. The signal reagent is coated with specific antibodies, which bind with the antibody-antigen complex, producing a black line. The presence of an upper black line (the control line) demonstrates the test has been performed correctly.

Materials Provided

The Malaria (p.f/p.v) Cassette Test kit contains the following items to perform the assay:

1. Test cassette individually foil pouched with a desiccant and a plastic sampling pipette
3. Sample Diluent
4. Package Insert

Materials Required But Not Provided

- Positive and negative controls

Storage and Stability

The kit must be stored at 2-30°C.

Warnings and Precautions

1. All Positive results must be confirmed by an alternative method.
2. Treat all specimen as though potentially infectious.
3. Wear protective gloves and clothing while handling specimens. Wash hands thoroughly afterwards.
4. Standard safety precautions in the handling of biohazard material should be observed in specimen handling.
5. Dispose of used lancets, pipettes and cassettes in designated biohazard disposal containers.
6. Device used for testing should be autoclaved before disposal.
7. Do not use kit material beyond their expiration dates.
8. Do not interchange reagents from a different lot of kit.

Specimen Collection and Storage

1. Collect whole blood specimen following regular clinical laboratory procedures.
2. Storage: A specimen should be refrigerated if not used the same day of collection. 0.1% of sodium azide can be added to specimen as preservative without affecting the results of the assay.

Test Procedure

1. Before testing, bring the device, sample diluent and specimen to room temperature.
2. Remove the test cassette from the sealed pouch.

Obtain Blood Sample



Figure 1

a. **Place selected finger flat on the tabletop.** With the thumb of your opposite hand. Massage or “milk” the selected finger, five or six times, to push blood to the tip (Figure 1).

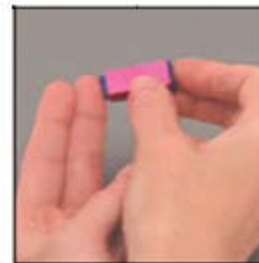


Figure 2

b. Place the raised end of the lancet firmly against the **side of the selected finger.**

Press the lancet against your finger until you hear a “click” You may feel a slight sting. (Figure 2).

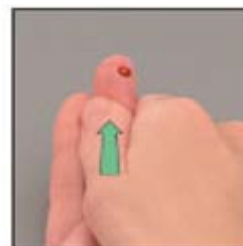


Figure 3

c. With the thumb of your opposite hand, massage or “milk” your finger until a **large drop of blood forms.** (Figure 3).

- ✓ Collect your blood using the plastic pipette provided

Assay Procedure

1. Dispense 1 drop (10µl) of whole blood to the “S” well of the test cassette using the plastic pipette provided, according to the illustrations above. (Figure 4)

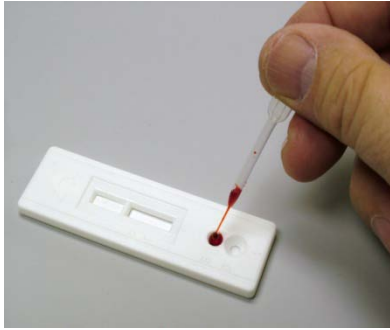


Figure 4

2. Add three drops of Sample Diluent to the “D” well after the specimen is added. (Figure 5)



Figure 5

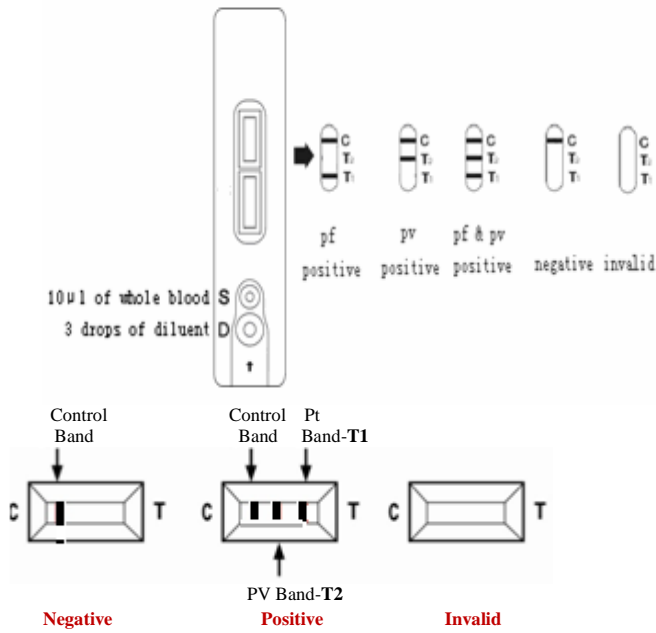


Illustration A

3. Interpret test results at 15 minutes.

Notes:

1. Applying sufficient amount of sample diluents is essential for a valid test result. If migration (the wetting of membrane) is not observed in the test window after one minute, add one more drop of diluent to sample well.
2. The positive results could appear as soon as 1 minute for a sample with high levels of Malaria.
3. Do not interpret results after 30 minutes.

Interpretation of the Test: (See Illustration A)

1. Positive: Control line and at least one Test line appear on the membrane. The appearance of T1 Test line indicates a Pf. HRP-II Positive result; the appearing of T2 Test line indicates a P. vivax specific pLDH Positive result, the appearing of both T1 and T2 Test lines indicate a Pf. HRP-II or P. vivax specific pLDH positive result. The lower the antigen concentration, the weaker the Test line.
2. Negative: Only the black Control line appears on the membrane. The absence of a Test line indicates a Negative result.
3. Invalid: There should always be a black Control line in the Control region, regardless of the test result. If the Control line is not seen, the test is considered Invalid. Repeat the test using a new test device.

Note: It is normal to have a slightly lightened control band with very strong Positive samples, as long as it is distinctly visible.

Performance Characteristics

The following data was generated from previously frozen whole blood samples and was determined by correlation to standard thick and thin smear microscopic examination with discrepancies evaluated via PCR. Retrospective study results are summarized below:

Site	Pos	Neg	Test Pos	Test Neg
India	66	86	64 (97%)	86 (100%)
Senegal	8	10	8 (100%)	10 (100%)
Varied Origin	48	53	46 (95.8%)	53 (100%)
South Africa	102	150	99 (97%)	149 (99.3%)
TOTAL	224	299	217 (96.9%)	298 (99.7%)

The Malaria Test did not cross-react with any of the following species of malaria: P.malariae, P.ovale.

Limitations

1. The assay should be performed in normal room temperature.
2. The test cassette should be used immediately after being taken from the package. Avoid exposing the test cassettes in the air for too long before use.
3. The test cassette may be stored under room temperature and dry conditions. If refrigerated, the cassettes should be brought to room temperature before testing.
4. Although the test is very accurate, a low incidence of false results may occur.
5. If questionable results are obtained, the test should be repeated on a fresh whole blood specimen using a new device.

Bibliography

1. WHO. World malaria situation in 1994. Part 1: Population at risk [J]. Wkly Epidemiol Rec, 1997, 72(36):269-74.
2. World Health Organization: WHO Expert Committee on Malaria, 20th Report. WHO Tech Report Series 892. WHO, 2000.
3. Quintana M, Piper R, Boling HL, et al. Malaria diagnosis by dipstick assay in Honduran population with coendemic *Plasmodium falciparum* and *Plasmodium vivax* [J]. Am J Trop Med Hyg, 1998 59(6): 868-871.
4. Tjitra E, Suprianto S, Dyer M, et al. Field evaluation of the ICT malaria P.f/P.v immunochromatographic test for detection of *Plasmodium falciparum* and *Plasmodium vivax* in patients with malaria in eastern Indonesia [J]. Clin a presumptive clinical diagnosis of Microbiol. 1999, 37 (8): 2412-2417.
5. Plamer CJ, Validum L, Lindo J, et al. rapid malaria TrancR Sne diagnostic test during anti Mrd Hve. 1999.93(5) Field evaluation of the OptiMAI Malaria therapy in Guyana [J]: 517-518.
6. Gilles HM: Management of Severe and Complicated Malaria. A Practical Handbook. WHO, 1991.
7. Goldsmith RS, Heyneman D: Tropical Medicine and Parasitology. Appleton & Lange, 1989.
8. Price DL: Procedure Manual for the Diagnosis of Intestinal Parasites. CRC Press, 1994.

Manufactured in the USA by:

HEALTH-CHEM DIAGNOSTICS LLC,

US FDA & ISO Certified Facilities

3341 SW 15th STREET, POMPANO BEACH, FL - USA

www.healthchemdiagnostics.com

Certified ISO CMDCAS 13485:2003



FM77504 - Quality Award

FDA Registration No.: 1048532