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
2. Sample Diluent
3. 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

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).

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).

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 an appropriate sampling device (not supplied). Only use the plastic pipette provided to apply the blood sample to the cassette well.

**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 below. *(Figure 4)*

![Figure 4]

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

![Figure 5]

3. Interpret test results at 15 minutes, (See Notes:).

**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:

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

In another study, three separate lots of HCD ONE STEP MALARIA Pf/Pv were tested by an independent reference laboratory on confirmed blood samples of Malaria Pf/Pv.

1044 patient whole blood samples consisting of 201 Positive cases and 843 Negative cases confirmed by microscopic examination (with a 99% confirmation rate). Testing was performed and evaluated in parallel.

Correlation on all cases exceeded 99.4%.
Limitations

1. The assay should be performed at 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


Manufactured by:
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