


**Symbol LEGENDS**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Explanation of symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>::</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>DV</td>
<td>In vitro diagnostic device</td>
</tr>
<tr>
<td>I/C</td>
<td>Store at 1°C - 40°C</td>
</tr>
<tr>
<td>C</td>
<td>Biological risk</td>
</tr>
<tr>
<td>Keep dry</td>
<td></td>
</tr>
<tr>
<td>Do not reuse</td>
<td></td>
</tr>
</tbody>
</table>

**References**


**Limitations and Interferences**

1. The test procedure, precautions and interpretation of results for this test must be followed when testing.
2. Anti-coagulants such as heparin, EDTA, and citrate do not affect the test result.
3. Interfering specimens like hemolytic (hemoglobin containing) samples, icteric (bilirubin containing) samples, lipaemic samples, Rheumatoid Factor containing samples do not affect the test results.
4. This test kit detects pLDH and HRP2 in patient’s whole blood and is useful as a screening procedure for malaria diagnosis.
5. Do not mix reagent of different lots.
6. The test is limited to the detection of antigen to Malaria Plasmodium sp. Although the test is very accurate in detecting pLDH and HRP2, a low incidence of false result can occur. 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.
7. Interprets false as a positive line. Repeat the test in case of false test line or if have any doubt for test line.
8. False negative results may arise because of hook effect due to very high parasitic samples. Repeat the test by using different dilution of same sample.
9. Repeat after 8 hours if suspicion malaria persists.
10. HRP2 may persist after treatment or passed P. falciparum infection.

**Assay Principle**

First Response® Malaria Ag. pLDH/HRP2 Combo Card Test is based on principle of immunochromatography in which nitrocellulose membrane is pre-coated with two monoclonal antibodies as two separate lines. One monoclonal antibody (test line PAN) is PAN specific to Lactate Dehydrogenase (pLDH) of the Plasmodium species (Plasmodium falciparum, P. vivax, P. ovale and P. malariae) and the other line (test line P.f.) consists of a monoclonal antibody specific to Histidine-Rich Protein 2 (HRP2) of the Plasmodium falciparum. When the test sample along with assay diluent flows through the nitrocellulose membrane, monoclonal antibodies conjugated with colloidal gold, which are PAN specific to pLDH and P. falciparum specific to HRP2 binds to Plasmodium antigens released from the lysed blood sample. These antigen-conjugated antibody complex moves through the nitrocellulose membrane and binds to corresponding immobilised antibody at test lines, which leads to the formation of colour band / bands indicating reactive results. The control band will appear irrespective of reactive or non reactive sample.

So, the First Response® Malaria Ag. pLDH/HRP2 Combo Card Test is “of additional value” in the differential diagnosis of Plasmodium falciparum and other Plasmodium species.

**Materials Provided**

- First Response® Malaria Ag. pLDH/HRP2 Combo Card Test kit contains the following items to perform the assay.
- Materials Required but Not Provided:
- Timer

**Storage & Stability**

First Response® Malaria Ag. pLDH/HRP2 Card Test should be stored at 1°C - 40°C. Do not freeze the kit or components. Assy Diluent (Carbonate buffer saline containing proteins and preservative)

**Precautions**

1. For in vitro diagnostic use only.
2. Test Devices and Assy Diluent of different lot must not be used.
3. Do not use the Test Device if the pouch is not intact.
**Test Procedure**

Ensure that the Device and Assay Diluent are at room temperature before starting the procedure.

1. Remove the Test Device and the sample pipette from the foil pouch. Label the Test Device with the patient identification number/ name. Place the test device on a flat surface.
2. Add 5 μl of whole blood into the Sample Well of respective Test Device.
3. Add two drops (60 μl) of Assay Diluent into the Assay Diluent Well.
4. Read the test result within 20 min. Do not interpret after 20 min.

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**Specimen Collection and Storage**

**[Collection by venipuncture]**

1. Collect the whole blood into the collection tube (containing EDTA/citrate/heparin) by venipuncture.
2. If specimens are not immediately tested, should be stored at 2 ~ 8°C upto three days. Using the specimen more than three days can cause non-specific reaction.
3. For storage periods greater than three days, freezing is recommended. Avoid repeated freezing & thawing of specimen.

**[Collection using a lancet]**

1. Clean the area to be lanced with an alcohol swab.
2. If specimens are not immediately tested, should be stored at 2 ~ 8°C upto three days. Using the specimen more than three days can cause non-specific reaction.
3. For storage periods greater than three days, freezing is recommended. Avoid repeated freezing & thawing of specimen.

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**Sample Pipette**

Gently squeeze the bulb immersed in the blood up to the 5 μl mark.

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**Interpretation of the Test**

**Negative Result**

If only one color band appear, at control line 'C' as in the figure, the specimen is negative.

**Positive Results**

If two color bands appears, one at control line 'C' and the other at test line 'P.F.', indicates the specimen is Positive for P. falciparum (in case of low parasitemia). If all three color bands appears, one at control line 'C', and the other two at 'P.F.' and 'PAN' as in the figure, it indicate specimen is Positive for either P. falciparum or mix infection of P. falciparum with any of other PAN malaria species.

**Invalid Result**

If no color band appear, at control line 'C' within the stipulated time then the result is invalid.

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**Performance Characteristics**

(A) 1. The Performance of First Response® Malaria Ag. pLDH/HRP2 Combo Card Test was evaluated using an in-house panel of Positive and Negative Malaria samples. The Status of the samples was determined by microscopic examination. The results are as follows:

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of Samples Tested</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum Positive</td>
<td>100 100 0 0 100 100</td>
<td>-- 100 %</td>
<td>-- 100 %</td>
</tr>
<tr>
<td>P. vivax Positive</td>
<td>100 100 0 0 100 100</td>
<td>-- 100 %</td>
<td>-- 100 %</td>
</tr>
<tr>
<td>Malaria Negative</td>
<td>200 0 200 0 100 100</td>
<td>-- 100 %</td>
<td>-- 100 %</td>
</tr>
</tbody>
</table>

(A) 2. The Performance of First Response® Malaria Ag. pLDH/HRP2 Combo Card Test was evaluated at various external laboratories & institutes. The summary of reports is as follows:

<table>
<thead>
<tr>
<th>Name of the Institute</th>
<th>Year of Testing</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uganda Virus Research Institute, Uganda</td>
<td>2008 100 100</td>
<td>-- 100 %</td>
<td>-- 100 %</td>
</tr>
<tr>
<td>Uganda Virus Research Institute, Uganda</td>
<td>2008 97.00 96.7</td>
<td>100 99.5</td>
<td>-- 100 %</td>
</tr>
<tr>
<td>Institut Pasteur du Cambodge, Cambodge, 2010</td>
<td>100 100</td>
<td>-- 100 %</td>
<td>-- 100 %</td>
</tr>
</tbody>
</table>

B) Analytical sensitivity

The sensitivity of First Response® Malaria Ag. pLDH/HRP2 Combo Card Test for P. falciparum and PAN Malarial Species is comparable to microscopic observation with more than or equal to 200 parasites per μl of blood. The product has not been fully assessed for P. ovale & P. malariae and the sensitivity is expected to be low.

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**Precision**

Within-run and between-run precisions have been determined by testing of 10 replicates of three specimens: a negative, a low positive and a strong positive. The agreement between the test results and the expected results were 100%.