Although the test is very accurate in detecting HRP2, a low incidence of false results 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.

Interpret faint line as a Positive line. Repeat the test in case of very faint test line or if have any doubt for test line.

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.

Repeat after 8 hours if suspicion malaria persists.

HRP2 may persist after treatment or passed infection.

First Response® Malaria Ag. P. falciparum (HRP2) Card Test is intended for use by healthcare professionals and as qualitative screening in vitro diagnostic test for detection of P. falciparum specific HRP2 antigens. The test is intended for use with whole blood. The test is not automated and does not require any additional reagent. Reactive samples should be confirmed by additional tests such as Microscopic examination.

Introduce

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 mosquitoes. There are four kinds of Plasmodium species 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 into the blood and it infects red blood cells. The disease now occurs in 109 countries worldwide and it is estimated that around 250 million cases annually, leading to approximately 1 million deaths (WHO 2008). At present, malaria is diagnosed by looking for parasites in a drop of blood. Blood is put onto a microscope slide and stained so that the parasites are visible under a microscope.

Assay Principle

First Response® Malaria Ag. P. falciparum (HRP2) Card Test is based on principle of immunochromatography in which nitrocellulose membrane is pre-coated with one monoclonal antibody (test line P.f.) specific to Histidine-Rich Protein 2 (HRP2) of the Plasmodium falciparum. When the test sample along with assay diluent flows through the nitrocellulose membrane, second monoclonal antibodies specific for HRP2 Ag. conjugated with colloidal gold, binds to HRP2 antigens released from the lysed blood sample. This antigen-conjugated antibody complex moves through the nitrocellulose membrane and binds to the immobilised HRP2 specific monoclonal antibody at the test line, which leads to the formation of colour band indicating reactive results. The control band will appear irrespective of reactive or non reactive sample.

So, the First Response® Malaria Ag. P. falciparum (HRP2) Card Test is designed for the diagnosis of Plasmodium falciparum.

Materials Provided

The First Response® Malaria Ag. P. falciparum (HRP2) Card Test kit contains the following items to perform the assay.

Reagents

Test Device with sample pipette and desiccant (Test device enclosed nitrocellulose test strip on which test and control lines are coated).

Assay Diluent (Carbonate buffer saline containing proteins and preservative).

Accessories

Instructions for use

Alcohol Swab

Sterile lancet.

Materials Required but Not Provided: Time: Storage & Stability

First Response® Malaria Ag. P. falciparum (HRP2) Card Test should be stored at 4 - 40°C. Do not freeze the kit or components. Assay diluent (opened & unopened) & the unopened Test Device are stable until the expiry date printed on the label, when stored at room temperature 4 - 40°C. The test device is sensitive to humidity and heat. Perform the test immediately after removing the Test Device from the foil pouch. The shelf life of the kit is as indicated on the outer package. Do not use Test Device and Assay Diluent beyond the date of expiry.

Precautions

1) For in vitro diagnostic use only.
2) Test Devices and Assay diluent of different lot must not be used.
3) Do not use the Test Device if the pouch is not intact.
4) Do not use the Lancet if the seal is broken.
5) Check the desiccant for saturation, immediately after opening the pouch.
6) Do not smoke, eat or drink while handling specimens and performing a test.
7) The test device, alcohol swab, lancet and sample pipette are intended for single use only.
8) Follow the assay procedure strictly, deviation will invalidate the results.
9) Perform the test by using kit assay diluent, any other diluent or fluid will invalidate the results.
10) Do not touch the tip of Assay Diluent bottle, it might contaminate Assay Diluent.
11) Wear protective gloves while handling specimens. Dispose off used gloves as biohazard waste. Wash hands thoroughly afterwards.
12) Avoid splashing or aerosol formation.
13) Clean up spills thoroughly using an appropriate disinfectant.
14) Decontaminate and dispose off all used specimens, test devices, alcohol swabs, lancets and sample pipette as a infectious waste, in a biohazardous waste container. Dispose off used lancet after recapping it properly.

**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) Squeeze the end of the fingertip and pierce with a sterile lancet provided.
3) Wipe away the first drop of blood with sterile gauze or cotton.
4) Take a sample pipette provided, while gently squeezing the bulb, immerse the open end in the blood drop and then gently release the pressure to draw blood into the sample pipette upto the 5μl mark.

**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 5μl of whole blood into the Sample Well of respective Test Device.
3) Add two drops (60μl) of Assay diluent into the Sample Diluent Well.
4) Read the test result within 20 min. Do not interpret after 20 min.

**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 'T', indicates the specimen is Positive for P. falciparum.

**Invalid Result**
If no color band appears, at control line 'C' within the stipulated time then the result is invalid. The result is also invalid if a color band appears only at test line 'T'.

**Performance Characteristics**

(A) 1. The Performance of First Response® Malaria Ag. P. falciparum (HRP2) 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>FIRST RESPONSE® Malaria Ag. P. falciparum (HRP2) Card Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>P. falciparum Positive</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>P. falciparum Negative</td>
<td>200</td>
<td>0</td>
</tr>
</tbody>
</table>

(A) 2. The Performance of First Response® Malaria Ag. P. falciparum (HRP2) Card Test was evaluated at various external laboratories & institutes. The summary of reports are 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>Research Institute for Tropical Medicine, Philippines</td>
<td>2011</td>
<td>100 %</td>
<td>100 %</td>
</tr>
<tr>
<td>Omni Diagnostics, Ghana</td>
<td>2010</td>
<td>100 %</td>
<td>100 %</td>
</tr>
<tr>
<td>Institut Pasteur du Cambodge, Cambodia</td>
<td>2009</td>
<td>100 %</td>
<td>100 %</td>
</tr>
<tr>
<td>WHO Evaluation Round 1</td>
<td>2008</td>
<td>100 %</td>
<td>100 %</td>
</tr>
</tbody>
</table>

B) Analytical sensitivity - The sensitivity of First Response® Malaria Ag. P. falciparum (HRP2) Card Test for P. falciparum is comparable to microscopic observation with more than or equal to 200 parasites per μl of blood.

**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 Plasmodium falciparum HRP2 in patient’s whole blood and is useful as a screening procedure for malaria diagnosis.
5) Do not mix reagent of different lots.