INTENDED USE

EzDx™ Malaria Pv/Pf Test is a rapid in-vitro immuno-chromatographic immunoassay for the qualitative detection of malaria infection in human blood sample indicating differential diagnosis between *P. falciparum*, *HRP-II* (*Plasmodium falciparum*, histidine-rich protein II) and *Plasmodium vivax* species (*P. vivax*, *pLDH*). The test is intended for primary screening of malaria infection in symptomatic patients of all age groups including neonates and pregnant women. The test is intended for professional use and must be used by a trained personnel.

CLINICAL SIGNIFICANCE

Malaria is a serious, sometimes fatal, parasitic disease. It is characterized by fever with chills, anemia and is caused by *Plasmodium* parasite that is transmitted from one human being to another by the bite of infected Anopheles mosquitoes. Four species of *Plasmodium* parasite are responsible for malaria infections in human viz *P. falciparum*, *p.vivax*, *p.ovale* and *P. malariae*. Of these, *P. falciparum* and *P. vivax* are the most prevalent. Early detection and differentiation of malaria is of paramount importance due to incidence of cerebral malaria and drug resistance associated with *falciparum* malaria causing most of the morbidity and mortality worldwide.

PRINCIPLE

EzDx™ Malaria Pv/Pf test utilizes the principle of immuno-chromatography. It has the test strip coated with Monoclonal Anti pLDH *P. vivax* antibodies (test line Pv ) specific to the lactate dehydrogenase of *plasmodium vivax* species and the other (test line Pf) with Monoclonal Anti-HRP-II which is specific to the Histidine Rich Protein-II of *P. falciparum*. As the test sample flows through the membrane assembly of the device after addition of the buffer solution, the colored colloidal gold, anti-pLDH antibody and the anti-HRP-II antibody conjugate complexes with the lysed blood sample. The malarial antigens get immobilized on the respective test lines on the nitrocellulose membrane which leads to the formation of red/purple colored band/s. The unreacted conjugate and unbound complex if any moves further on the membrane and are subsequently immobilized at the control “C” region forming a red /purple band. This control band serves to validate the test performance and should always appear.

MATERIALS PROVIDED

A EzDx™ Malaria Pv/Pf kit contains the following items to perform the assay:
1. EzDx™ Malaria Pv/Pf Test Device individually foil pouched with a Desiccant.
2. Sample Applicator
3. Alcohol Swab
4. Lancet
5. Buffer Solution.

B Active ingredients of main components are:
- 1 test strip includes : Gold conjugate: Mouse monoclonal antibodies specific to pLDH of *Plasmodium vivax* - gold colloid, Mouse monoclonal antibodies specific to *PF* - HRP-II gold colloid, Test line Pv: Mouse monoclonal antibodies specific to *pfLDH* of *plasmodium vivax*, Test line Pf: Mouse monoclonal antibodies specific to *PF HRP-II*, Control line : Goat anti-mouse IgG.
- Buffer Solution: Casein (q.s), Triton X- 100(q.s).

MATERIALS REQUIRED BUT NOT PROVIDED

*Timer

PRECAUTIONS

1. Read this Insert carefully before carrying out the test and instruction must be followed exactly to get accurate results.
2. The Device is sensitive to humidity as well as to heat. Therefore take out the Device from sealed pouch just before carrying out the test.
3. Store the EzDx™ Malaria Pv/Pf kit at the temperature mentioned on the kit in a dry atmosphere.
4. Do not use the kit after the expiration date.
5. Do not mix reagents from different lots.
6. For In-Vitro diagnostic use only.
7. Wear protective gloves while handling samples and wash hands thoroughly after performing the test.
8. Dispose all the samples and kits properly as per the instructions after test in accordance with GLP.
9. Do not pipette reagents or blood sample by mouth.
10. Do not re-use the test.

11. Buffer Solution: It contains Sodium azide as a preservative, in case of contact with skin, wash immediately. Wear gloves and eye protective.

**SPECIMEN COLLECTION, STORAGE & PRECAUTION**

**Collection by venipuncture**
1. Collect the whole blood into the collection tube containing anticoagulant (EDTA, Citrate or Heparin) by venipuncture. Anticoagulant such as Heparin, EDTA and Citrate do not affect the test results.
2. If immediate testing is not possible then the samples may be stored at 2–8°C for up to 3 days. The sample should be brought to room temperature prior to use. Using the samples in the long term keeping more than 3 days can cause non-specific reactions.
3. Ideally samples when stored at 2–8°C, the whole blood samples should be used within 3 days.

**Collection with lancet**
1. Clean the fingertip area to be pricked with an alcohol swab.
2. Squeeze the end of the fingertip and pierce with the lancet.
3. Wipe away the first drop of blood with sterile gauze or cotton.
4. While gently squeezing the disposable Sample Dropper (5µl) provided, immerse the open end in the blood drop and gently release to draw 5µl of blood up to the indicated mark with even pressure.

   OR

   Take a disposable sample loop (5µl) provided, dip the circular end of the loop into the blood sample and to lead the blood sample carefully place correctly the circular end of the loop into the “Sample Port 1(S)” on the test device.

**Precaution**
1. Use separate sample Dropper (5µl) / Sample loop (5µl) or fresh pipette tip for each sample to avoid cross-contamination and erroneous results.
2. Do not use any other specimen than whole blood.

**TEST PROCEDURE**

1. Allow EzDx™ Malaria Pv/Pf kit components and specimens to attain room temperature.
2. Open the pouch and take out the Device from it.

   **NOTE:** The Device contains a blue control line made up of water soluble dye that disappears as the test runs.
3. Tighten the bottle cap of the Buffer solution provided with the kit in the clockwise direction to pierce the Dropper Bottle nozzle. Hold the buffer bottle vertically - this ensures that drops contain the correct volume of buffer.
4. Evenly mix the anticoagulated blood sample by gently swirling and then dip the sample Applicator (5µl) into the blood sample to draw 5µl of blood as show in the figure.

   OR

   Incase of finger prick, clean the fingertip with an alcohol swab dry completely; prick the fingertip with a “Single Use Lancet” Wipe away the first drop of blood with sterile gauze or cotton; with the help of the Sample Applicator (5µl), provided, draw 5µl of blood as show in the figure.
5. Load 5µl of blood into the “Sample Port 1(S)”.
6. Add 4 drops (110µl ± 5µl) of Buffer Solution into the “Buffer Port 2(B)” on the Test device.
7. Read the result at the end of 20 minutes.
8. Interpret the result. Refer for figure for interpretation of the result.

**CAUTION:** Do not read test after 30 minutes, since it may give incorrect results.

**INTERPRETATION OF THE RESULTS**

Whole blood samples may cause red background to appear in the result window. If this is not masking the test line, the result remains valid.

**NEGATIVE:**

Only the control band appears. Negative result indicates no malaria antigens present in the blood sample, indicating no malaria infection or the number of malaria antigens present in the blood sample is below the detectable range.

**POSITIVE:**

1. *P. falciparum* Positive: Two bands (“Pt” Test line and “C” Control line) appears within the result window indicates infection with *P. falciparum*.

2. *P. vivax* Positive: Two bands (“Pv” Test line and “C” Control line) appears within the result window, indicates infection with *P. vivax*.

3. Mixed Infection: Three bands (“Pt” test line, “Pv” test line and “C” control line) appears within the result window, indicate mixed infection of *P. falciparum* and *P. vivax*.

The shade of color / Intensity of band may vary, but it should be considered positive whenever there is even a faint line.

**INVALID:**

If control band does not appear, the test is invalid. In this case, please repeat the test using a fresh device, and follow the test procedure exactly.

**STORAGE & EXPIRATION**

1. EzDx™ Malaria Pv/Pf kit should be stored between 2° to 40°C(36°-94°F).

   **DO NOT FREEZE.**

2. Expiry date of this kit is as indicated on the kit carton as well as on individual pouches.

**LIMITATIONS OF THE TEST**

EzDx™ Malaria Pv/Pf is designed for primary screening of malaria infection. Although the test is accurate in detecting HRP-II specific to *P.falciparum* or pLDH specific to *Plasmodium vivax* in blood sample, 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.

**PERFORMANCE CHARACTERISTICS**

The EzDx™ Malaria Pv/Pf Rapid Malaria Ag Detection Kit has been tested with Malaria Positive and Negative clinical samples tested by microscopic examination of whole blood. The results obtained are as follows:

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total No. Of Samples tested</th>
<th>Test Results of EzDx™ Malaria Pv/Pf Rapid Malaria Ag Detection Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vivax</em> Positive</td>
<td>120</td>
<td>118</td>
<td>02</td>
<td>98.33</td>
</tr>
<tr>
<td><em>P. falciparum</em> Positive</td>
<td>90</td>
<td>88</td>
<td>02</td>
<td>97.77</td>
</tr>
<tr>
<td>Mixed Infection</td>
<td>13</td>
<td>13</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Malaria Negative</td>
<td>190</td>
<td>03</td>
<td>187</td>
<td>–</td>
</tr>
</tbody>
</table>

**REFERENCES**


**DISCLAIMER**

Every precaution has been taken to ensure the diagnostic ability and accuracy of this product. This product is used outside of the control of the manufacturer and the distributor and the result may accordingly be affected by environmental factors and / or user error. A person who is the subject of the diagnosis should consult a doctor for further confirmation of the result.

**WARNING**

The Manufacturers and Distributors of this product shall not be liable for any loses, liability, claims, costs or damages whether direct or indirect or consequential arising out of or related to an incorrect diagnosis, whether positive or negative, in the use of this product.

For any complaint/query and suggestions: Customer Care No.: +91 22 25830326

**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>PACK SIZE</th>
<th>PRODUCT CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 Tests / Kit</td>
<td>RK MAL 003 - 10</td>
</tr>
<tr>
<td>25 Tests / Kit</td>
<td>RK MAL 003 - 25</td>
</tr>
<tr>
<td>50 Tests / Kit</td>
<td>RK MAL 003 - 50</td>
</tr>
</tbody>
</table>

**SYMBOL LEGENDS**

Manufactured in India by:

ADVY CHEMICAL PVT. LTD.
E-mail: info@advychemical.com Website: www.advychemical.com

Date Issued: 2017-04
EzDx™ Malaria Pv/Pf Test Procedure

1. FIRST, read carefully the Product Insert on how to use the EzDx™ Malaria Pv/Pf kit.

2. Now open the kit and look for the following.

   1) Test device individually foil pouched with a desiccant.

   2) Buffer Solution

   3) Product Insert

   4) Sample Applicator

   5) Lancet

3. Next, look at the expiry date on the back of the pouch. Use another kit, if expiry date has passed.

4. Clean the patient’s finger. The alcohol MUST be dry before pricking, or test may not work.

5. Prick the patient’s finger with the lancet to get blood. Wipe away the first drop of blood with sterile gauze or cotton.

6. Take 5µl blood using the Sample Applicator Provided.

   Sample Dropper (5µl)

   OR

   Sample Loop (5µl)

7. Add 5µl blood into Sample Port "1(S)".

8. Add Buffer Solution into the Buffer Port "2(B)" of the test device.

9. INTERPRET TEST RESULTS AT THE END OF 20 MINUTES.

   CAUTION: Do not read test after 30 minutes, since it may give incorrect results.

10. Interpretation of Test Results.

    | Negative For Malaria | Positive For Pv | Positive For Pf | Mixed Infection |
    |----------------------|-----------------|-----------------|-----------------|
    | EzDx Malaria Pv/Pf Test | EzDx Malaria Pv/Pf Test | EzDx Malaria Pv/Pf Test | EzDx Malaria Pv/Pf Test |
    | Only one red purple band appears in the result window | In addition to Control band, red-purple PV band appears in the result window | In addition to Control band, red-purple PF band appears in the result window | In addition to Control band, red-purple PV and PF band appears in the result window |

Content:

1 Test Device
1 Desiccant
1 Test
1