INTENDED USE
Wondfo One Step Malaria P.f/Pan Whole Blood Test Cassette is a rapid self-performing, qualitative, two site sandwich immunoassay, utilizing whole blood for the detection of P.falciparum specific histidine rich protein-2 (P.f HRP-2) and Pan specific pLDH. The test may also be used for differentiation of P.falciparum and other malarial species in whole blood samples and for the follow up of anti-malarial therapy. For in vitro diagnostic use only. For healthcare professional use only.

SUMMARY
Malaria remains one of the most serious tropical and subtropical diseases in many countries of the world. It is rampant in most areas of the tropics. Malaria is caused by a parasite that is transmitted from one person to person by the bite of infected Anopheles mosquitoes. There are four kinds of malaria that can infect humans: Plasmodium falciparum, P.vivax, P.ovale and P.malariae. Malaria also has been reported from blood transfusions or congenitally from mother to child. It is estimated to affect more than 500 million people causing between one and three million deaths every year.

PRINCIPLE
Wondfo One Step Malaria P.f/Pan Whole Blood Test utilizes the principle of immunochoromatography. As the test sample flows through the membrane assembly of the device after addition of the buffer, the colored monoclonal P.f specific HRP-2, Pan specific pLDH colloidal gold conjugate antibodies complexes the proteins in the lysed sample. This complex moves further on the membrane to the test region where it is immobilized by the P.f specific HRP-2 antibody / Pan specific pLDH which will lead to a formation of purple color band/s. While both the bands will appear at the test region in falciparum positive samples, only one band would appear in non-falciparum malaria positive sample. Absence of this color band/s in the test region indicates a negative test result. To serve as a procedure control, a colored line will appear at the control region (C), if the test has been performed properly.

PRECAUTIONS AND WARNINGS
1. This kit is for in vitro use only. Do not swallow.
2. Discard after first use. The test cannot be used more than once.
3. Do not use test kit beyond the expiration date.
4. Do not use test kit if the pouch is punctured or not well sealed.
5. Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious waste, in a biohazard container.
6. Wear protective gloves while handling specimens. Wash hands thoroughly afterwards. Avoid splashing or aerosol formation. Clean up spills thoroughly using an appropriate disinfectant.
8. DISPOSAL OF THE DIAGNOSTIC: The used-device has the infectious risk. The process of disposing the diagnostic must follow the local infectious disposal law or laboratory regulation.

CONTENT OF THE KIT
1. 25 Individual pouches, each containing:
   ■ Test Device
   ■ Desiccant pouch
   ■ 5µl sample dropper
   ■ 5 ml clearing buffer in a dropper bottle
   ■ Leaflet with instructions for use.
Optional: not included in the standard kit package, please contact your local distributor for ordering.

STORAGE AND STABILITY
1. Stored at 4ºC to 30ºC in the sealed foil pouch up to the expiration date.
2. Keep away from sunlight, moisture and heat.
3. DO NOT FREEZE.

SPECIMEN COLLECTION AND PREPARATION
Collection by venipuncture
1. Collect the whole blood into the collection tube (using the suitable anti-coagulant) by venipuncture.
2. If specimens are not immediately tested, they should be refrigerated at 2ºC – 8ºC. For storage periods greater than three days, freezing is recommended. They should be brought to room temperature prior to use.

Collection using a lancet
1. Select the finger for puncture, usually the side of the third or fourth finger. Clean the area to be lanced with an alcohol pad. Allow the finger to dry thoroughly.
2. Using a sterile lancet, puncture the skin just off the centre of the finger pad. Hold the finger downward. Apply gentle pressure beside the point of the puncture. Avoid squeezing the finger to make it bleed. Wipe away the first drop of blood with a sterile swab. Allow a new drop of blood to form. If blood flow is inadequate, the subject’s finger may have to be gently massaged at the finger base to produce a droplet of sufficient volume. Avoid ‘milking’ the finger.
3. Take a dropper provided, while gently squeezing the tube, immerse the open end in the blood drop and then gently release the pressure to draw blood into the dropper.

TEST PROCEDURE
1. Remove the test cassette from the foil pouch by tearing at the notch and place it on a level surface.
2. Slowly add 5 µl of whole blood to the sample well (A) and then add 4 drops (90~100ul) of clearing buffer to the buffer well (B).
3. As the test begins to work, you will see purple color move across the result window in the center of the test device.
4. Wait for 15 minutes and read results. Do not read results after 30 minutes.

INTERPRETATION OF RESULTS
Positive (+)
1. The presence of two color band ("T1" and "C") indicates a positive result for a infection with P. falciparum
2. The presence of two color bands ("T2" and "C") indicates a positive result for Non-\textit{P. falciparum} (\textit{P. vivax, P. malariae} or \textit{P.ovale}) infection.

3. The presence of three color bands ("T1", "T2" and "C") indicates a positive result for \textit{P. falciparum} monoinfection or "mixed" infection.

**Negative (-)**
The presence of only one band ("C") within the result window indicates a negative result.

**Invalid**
If the color band ("C") is not visible within the result window after performing the test, the result is considered invalid. The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be re-tested.

**QUALITY CONTROL**
A procedural control is included in the test. A colored line appearing in the control region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Good laboratory practice recommends the use of the control materials. Users should follow the appropriate federal state, and local guidelines concerning the frequency of assaying external quality control materials.

**LIMITATIONS OF PROCEDURE**
The test is limited to the detection of \textit{P.falciparum} specific histidine rich protein-2 (PfHRP-2) and Pan specific pLDH. Although the test is accurate in detecting antigens of \textit{Malaria P.f/Pan}, a low incidence of false results can occur. Other clinically available test 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**

1. Wondfo One Step Malaria P.f/Pan Whole Blood Test have a sensitivity of >90% at densities above 50-100 parasites /ul blood.

2. The presence of only one band ("C") within the result window indicates a negative result.

3. Wondfo Rapid Test have a sensitivity of >90% at densities above 50-100 parasites /ul blood.

<table>
<thead>
<tr>
<th>Test Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
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</thead>
<tbody>
<tr>
<td>Wondfo Rapid Test</td>
<td>96.0%</td>
<td>97.0%</td>
</tr>
<tr>
<td>Microscopic examination</td>
<td>-</td>
<td>-</td>
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</tbody>
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**Table 1** Result of the sensitivity and specificity between (Malaria P.f):

**Table 2** Result of the sensitivity and specificity between (Malaria Pan):

<table>
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<tr>
<th>Test Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
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<td>96.4%</td>
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<tr>
<td>Microscopic examination</td>
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**BIBLIOGRAPHY OF SUGGESTED READIN**