Reszon Diagnostics International Sdn. Bhd.
Revongen Corporation Center, No. 12A, Jalan TP5, Taman Perindustrian UEP, 47600 Subang Jaya, Selangor, Malaysia.

Malaria Rapid (Pan/ Pf)

(A rapid test for the differential detection of *Plasmodium falciparum* and/or *Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae* antigen in whole blood)

INTENDED USE

Malaria Rapid (Pan/ Pf) is an immunochromatographic assay designed for the qualitative detection and differentiation of *Plasmodium falciparum* and/or *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* antigen in whole blood. It is intended to be used as *in vitro* diagnostic of malaria. The results obtained should not be the sole determinant for clinical decision.

SUMMARY AND EXPLANATION OF THE TEST

Malaria is one of the world's most prevalent parasitic diseases and ranks third in the world among major infectious diseases in terms of mortality. The protozoal parasites that cause malaria are from the Plasmodium genus. Four species of Plasmodium protozoa cause malaria: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*. Transmitted principally by the Anopheles mosquito, malaria infections may also occur from contacting infected blood, such as from blood transfusions.

P. falciparum accounts for the majority of infections and is the most lethal. *P. vivax*, *P. malariae* and *P. ovale* cause a less severe form of malaria with intermittent fever which is usually neither debilitating nor fatal. Classic symptoms of malaria include fever, headaches, chills, vomiting, shivering and convulsions. In some rare forms of *P. falciparum* malaria, chills and fever may be absent and the patient may present with delirium or coma. Remission periods can last from a few weeks to several months. Severe anemia is often attributed to the cause of death from a malaria infection.

Malaria is a curable disease with a host of drugs that can be used in both its treatment and prevention. Two of the best known and most commonly used are chloroquinine and quinine. The early detection of *P. falciparum* malaria is of great importance due to rising levels of drug resistance now being associated with this disease.

Malaria Rapid (Pan/ Pf) provides an excellent methodology for specifically detecting and differentiating of the circulating malaria antigens in whole blood. Additional advantages includes:

- fast, simple and reliable
- simple to perform and no additional sample preparation required
- no special equipment is needed
- results are easy to interpret
- minimal sample volume used

PRINCIPLE OF THE TEST

Malaria Rapid (Pan/ Pf) is an indirect solid-phase immunochromatographic assay. The test uses antibodies that are specific for the histidine-rich protein 2 antigen (HRP-2) of malaria *P.f.* and Plasmodium lactate dehydrogenase (pLDH) for the detection of all malaria Plasmodium species.

Whole blood (3 µl) is applied to the sample well where the red blood cells are lysed with a specially formulated buffer solution. The conjugate pad that is contained in the test is impregnated with blue latex that has an anti-HRP-2 antibody coupled to it, along with second blue latex that has an anti-pLDH antibody coupled to it. The conjugate pad is also impregnated with purple latex that is coupled to a control antibody. An additional anti-HRP-2 antibody is immobilized on the test at the "*P.f.*" test line region. Another anti-pLDH antibody is immobilized on the test strip at the "All test line" region. Finally, a control material is immobilized at the control line region.

When a positive sample is applied to the sample port, malaria antigen in the sample contacts the latex-labeled antibody and binds to it. A washing reagent is then added to the buffer well. As the liquid flows along the length of the device, any antigen-latex complexes also migrate with the liquid. These complexes are captured by their respective antibodies at the *P.f.*, All and control line regions. If a sample contains *P.f.* antigen, a blue line will form in the *P.f.* test region and may or may not form in the All test region, depending on the titer of the antigen present. If the sample contains *P.v., P.o.* or *P.m.* antigen, a test line will form in the All test region. If no malaria antigen is present, a blue line will not form in either *P.f.* or All test regions. A purple control line will always appear in the control region if the test has been performed properly.

PERFORMANCE CHARACTERISTICS

Sensitivity and specificity for *Malaria Rapid (Pan/ Pf)* on *P. f* test are 100% and 98% respectively; while sensitivity and specificity on pLDH test are 97% and 98% respectively.

REAGENTS AND MATERIALS SUPPLIED

1. *Malaria Rapid (Pan/ Pf)* cassette (25 pieces packed in individually sealed aluminium pouch)
2. One bottle of assay buffer
3. One copy of instruction manual (product insert)

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Lancets, sample collection capillaries and disinfecting sterile wipes
2. Sample dispensing apparatus such as pipettes
3. Clock or timer

STORAGE AND STABILITY

Store at 4-30 ºC, do not freeze. Keep the test device sealed until used. Keep away from direct sunlight, moisture and heat.

The test has been found to be stable for up to 24 months from the date of manufacture when stored between 4- 30℃. The expiration date of each test can be found on the pouch label. No component or reagent of the test should be used beyond its printed expiration date.

WARNINGS AND PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2. This product insert must be strictly followed in order to produce accurate test results.
3. Keep the test device sealed until use. Once the device pouch has been opened, the test device must be used immediately.
4. All test devices, reagents and specimens must be at room temperature (15-30℃) before running the assay.
5. Do not use the test device and buffer beyond the expiration date.
6. Be careful not to touch the tip of the buffer bottle to the sample tube when adding buffer to the tube. This will greatly minimize the likelihood of contaminating the buffer reagent
7. The wash solution contains a low concentration of sodium azide as a preservative (less than 0.01 %). Sodium azide is toxic. Do not drink this buffer. Sodium azide may also react with lead and copper in plumbing to form explosive compounds. If you dispose of this buffer down a drain, flush the drain with excess amounts of water to minimize the accumulation of potentially explosive metal-azide compounds.
8. Handle all specimens as being potentially infectious. Dispose all materials that come in contact with the specimen as infectious waste.
9. Do not reuse test device or buffer.

LIMITATION OF THE TEST

1. This product is designed for use with human blood only.
2. This test detects the presence of malaria antigens in whole blood and should not be used as the sole criterion for the diagnosis of a malaria infection.
3. Strict adherence to the test procedure is required. Do not re-use negative devices. Do not adulterate the buffer reagent.
4. HPR-2 tests may give positive malaria results for up to 2 weeks following therapy and parasite clearance as confirmed by microscopy.
5. pLDH tests may give positive malaria results for several days following therapy and parasite clearance as confirmed by microscopy.
6. The test is a qualitative assay and cannot be used to monitor therapy or to estimate the titer of the infection.
7. The results obtained should only be interpreted in conjunction with other diagnostic results and clinical information. If the test result is negative and malaria infection suspicion still exists, additional follow-up testing using other clinical methods is recommended.
8. A negative result at any time does not preclude the possibility of an early malaria infection.
9. A final diagnosis should be based on these test results in conjunction with other clinical and laboratory findings.

WARRANTY AND LIMITED LIABILITY

The performance characteristics stated were obtained by using the assay procedure in this insert. Failure to follow the assay procedure may derive inaccurate results. In such event, the manufacturer disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and the fitness for use.
The manufacturer will not be liable for any damage caused by misuse, improper handling and storage, non-compliance with warnings and procedures, damage caused by events occurring after the product is released, failure to ensure the product is in proper condition before use, or any warranty given by independent distributor.

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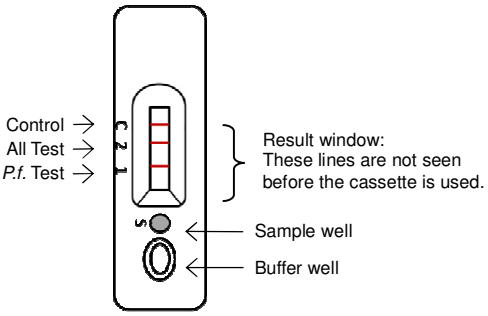
SAMPLE COLLECTION AND PREPARATION

1. Handle all specimens as capable of transmitting infectious diseases. Dispose of all materials that come in contact with the specimen as infectious waste.
2. Specimens should be collected aseptically by fingerstick or venipuncture according to standard methods. The use of grossly lipemic or turbid samples should be avoided.
3. Whole blood samples should be used immediately, if possible.
4. Use the collection capillary provided to deliver approximately 3 µl sample or collect venous blood into EDTA tubes. To obtain capillary blood, puncture a finger, heel or other appropriate site. First cleanse the area with a disinfecting sterile wipe. Use a lancet to puncture the skin. Allow a blood droplet to form. Touch the collection capillary to the blood droplet and transfer to the test strip immediately. To collect venous blood, use the standard venipuncture procedure and collect blood into an EDTA tube. If the test cannot be performed immediately, the blood may be stored for up to three days at 2℃ to 8℃.

ASSAY PROCEDURE



1. Bring test cassette and Lysing/Wash buffer to room temperature (if precipitates are noted in the chase buffer reagent, shake the bottle vigorously and allow to warm up further).
2. Gently tear open the pouch and remove the test cassette. Lay the test device on a clean, flat work surface. Label the test cassette with the sample name.
3. Using a sterile lancet and clean sample capillary, collect blood by puncturing an accessible site (e.g., finger or heel). Allow a blood droplet to form at the puncture site and touch the tip of the capillary to the blood droplet. Allow blood to fill about 3/4 of the capillary. Alternatively, 3 µl of EDTA venous blood may be used. Ensure that the blood sample warms to room temperature prior to use.
4. Transfer the blood sample from the capillary tube to the test device by holding the capillary vertically and gently touching the full end against the pad within the sample addition well until all of the blood has been transferred. Discard the capillary properly. If using a micro-pipetter, slowly apply 3 µl of blood to the sample pad.
5. Immediately add one drop of the Lysing/Wash buffer reagent to the same sample well on top of the whole blood.
6. Add five drops (drop by drop) of the Lysing/Wash buffer reagent to the buffer well.
7. Using a timer, allow the reaction to proceed for 15 minutes. Do not pick up the device during this time.
8. When the 15-minute period is over, read the results. If there is still a reddish background, lay the device flat on the work surface and wait an additional 15 minutes. The results may be read from 15 to 30 minutes. Do not read results after 60 minutes.

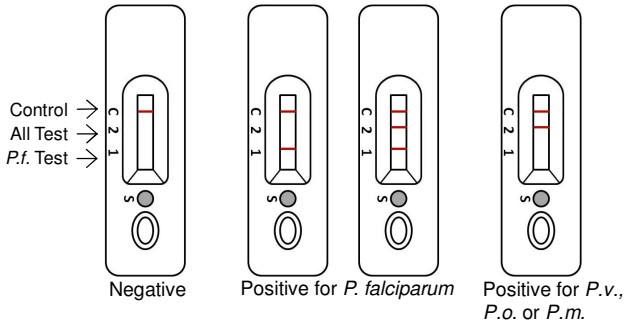
QUALITY CONTROL

1. Positive and negative controls are not included and are optional.
2. If the control line at position C does not become visible, the test is invalid and the test must be repeated. Positive samples will have additional coloured band at zone *P.f.* and/or All Test.

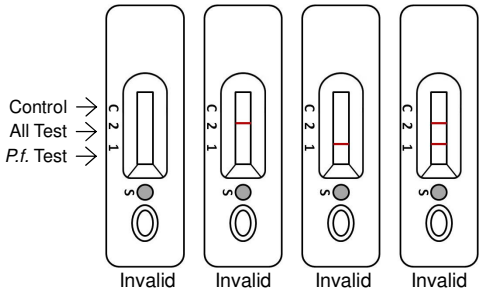
INTEPRETATION OF RESULTS

This test contains two test result lines: A test line that solely detects Malaria *P.f.* and a test line that detects all four Malaria Plasmodium species: *P.f., P.v., P.o.* and *P.m.*

The background of the strip should be pinkish-white, not red, prior to confirming a negative result. Positive results may appear as early as 5 minutes. Negative results must be confirmed after or at 30 minutes. Results should not be read after 60 minutes.



1. **Negative**:
   Only control line (C) is visible. No malaria antigens were detected.
2. **Positive for *Plasmodium faciparum*:**
   Coloured bands appear at the control line (C) and test line(s) at *P.f.* zone, or both *P.f.* & All Test zone.
3. **Positive for *Plasmodium vivax*, *Plasmodium malariae* or *Plasmodium ovale*:**
   Coloured bands appear at the control line (C) and test line at All Test zone. The test cannot distinguish between these malaria subtypes.



Invalid: Control line (C) is absent. If this occurs, the assay should be repeated using a new test cassette.

Plasmodium lactate dehydrogenase (pLDH) is secreted by all four Plasmodium species. Its presence usually indicates a malaria infection. Occasionally, residual lactate dehydrogenase (pLDH) may be detected for several days following elimination of the parasite by anti-malarial treatment. The diagnosis of Malaria should be made using the results of this test together with the other clinical and laboratory findings.

REFERENCES

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3. Beadle, C, et.al. Diagnosis of malaria detection of Plasmodium falciparum HRP-II antigen with rapid dipstick antigen capture assay. Lancet (1994), 343: 564-568.
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5. World Health Organization Fact Sheet (1998, Malaria, No.94).
6. CDC/NIH Guidelines. Biosafety in Microbiological and Biomedical
7. Laboratories. 2nd Edition, 1988.
8. Siti-Strong. Diagnosis, prevention, and treatment of tropical disease, 7th ed., Philadelphia, The Ablakiston Company.

ORDER INFORMATION

Product Code	***Description***	***Packing Size***
ML-RD0101	Malaria Rapid (Pan/ Pf)	25 tests / kit

MANUFACTURER

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