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Malaria P.f/P.v. Rapid Test (Cassette)

For *in vitro* diagnostic use only.
For export use only.

REF

Cat.# R6-538

IVD

INTENDED USE

Immunospec Malaria Rapid Test for differential plasmodium infection is a rapid immunochromatographic assay for the qualitative detection of plasmodium lactate dehydrogenase (pLDH) in human blood as an aid in the diagnosis of Malaria infection. Malaria Rapid Test has employed a new generation of high affinity anti-pLDH antibodies for the development of a highly sensitive assay system. This test cassette is intended for *in-vitro* diagnostic use only.

SUMMARY

Four *Plasmodium* species cause human disease, *P. falciparum* (*P.f.*), *P. vivax* (*P.v.*), *P. ovale* (*P.o.*), and *P. malariae* (*P.m.*). Of these, *P. falciparum* remains by far the most severe form, is resistant to many common drugs, and is responsible for nearly all deaths from malaria (1). Diagnosis of malaria (2-6) has traditionally been done by microscopic examination of blood. Microscopy, however, is time-consuming, particularly in patients with low parasitemias, since the microscopist must carefully scan 50-100 fields to ensure a slide is negative. Other diagnostic methods have included fluorescence microscopy, including the Quantitative Buffy coat method using acridine orange to stain intracellular parasites, and the polymerase chain reaction (PCR) to detect parasite DNA from whole blood. *Currently available antigen detection assay kits from other manufacturers are not capable of detecting all malaria species (especially P. ovale, which is predominant in Africa).* Immunospec has developed a pLDH based rapid diagnostic tests for malaria in the lateral-flow format that can be used with finger-stick or venous blood. *Presence of pLDH can be used for monitoring therapeutic efficacy as only dividing cells produce the LDH.*

TEST PRINCIPLE

The Malaria p-LDH antigen test contains a membrane strip, which is pre-coated with two monoclonal antibodies as two separate lines across a test strip. One monoclonal antibody (test line 1; bottom line) is pan-specific to the lactate dehydrogenase of all plasmodium (*P. falciparum*, *vivax*, *malariae*, *ovale*) and another monoclonal antibody (test line 2; below the control line) is specific to the lactate dehydrogenase of *P. falciparum* species. A conjugate pad is dispensed with a different pan-specific monoclonal antibody and recognizes all plasmodium. Once the blood sample is applied, the blood and the anti-pan LDH antibody gold mix migrate upward on the membrane. Depending on what type of plasmodium infected RBC is used, red line(s) will be generated. Reaction occurs in both T1 and T2 for *P.f.* infection. Reaction occurs in T1 for non *P.f.* infection, which includes *P.v.* The Malaria plasmodium p-LDH Test is designed for the differential diagnosis between *Plasmodium falciparum* and the other *Plasmodium* species. Presence of this red line indicates a positive result, while its absence indicates a negative result. Regardless of the presence of pLDH, as the mixture continues to migrate across the membrane to the immobilized control region (C line) region, a

red line at the control line region will replace the blue line present on the test strip prior to running the assay. The presence of this red line serves as verification for sufficient sample volume and proper flow and as a control for the reagents.

REAGENTS AND MATERIALS SUPPLIED

1. Twenty-five (25) test device, individually pouched.
2. One vial of Chase Buffer solution.

Optional:

- Extra assay Buffer
- Sample Pipette (accurately measures 5 µl samples)
- Lancet
- Alcohol Swab
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PRECAUTIONS

- For *in-vitro* diagnostic use only. Do not use after expiration date.
- Handle all blood and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of sera and used kits.
- Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly when finished.
- Avoid all contact between hands and eyes or mucous membranes during testing.
- Do not eat, drink or smoke in the area where the sera and kits are handled.
- Chase Buffer contains a preservative; avoid all possible contact with skin and mucous membranes.
- Clean up spills thoroughly using an appropriate disinfectant.

STORAGE

The sealed pouch containing the test cassette is designed to be stored at room temperature (8°C-30°C) for the duration of its shelf life. The bottle containing the Chase Buffer is designed to be stored at room temperature for the duration of its shelf life. Exposure to temperatures over 30°C can impact the performance of the test and should be minimized. The strips should not be frozen. The test should be used within 15 minutes after removal from the pouch to prevent exposure to humidity.

SPECIMEN COLLECTION AND PREPARATION

[Collection by venipuncture]

1. Collect the whole blood into the collection tube (containing EDTA, citrate or heparin) by venipuncture.
2. **For best results (APPLIES TO ALL COLLECTION PROCEDURES)**
 - a. Use freshly drawn and fully un-coagulated and easy flowing blood samples.
 - b. If not used right away, samples should be refrigerated at 2-8°C. Un-coagulated blood samples more than 2-3 days old SHOULD NOT be used (samples should be brought to room temperature prior to use). No clot should be present. Clots will impair the test results.
 - c. If samples are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.

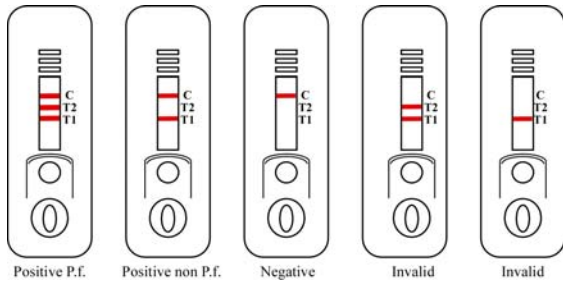
[Collection using a lancet]

1. Clean the area to be lanced with an alcohol swab.
2. Squeeze the end of the fingertip and pierce with a sterile lancet.
3. Wipe away the first drop of blood with sterile gauze or cotton.
4. Using the dropper provided, while gently squeezing the tube, immerse the open end of the tube into the blood drop and then gently release the pressure to draw blood into the dropper.

PROCEDURE

- 1) Add 5 µl of whole blood into Sample Well using a single channel micropipette, or a sample pipette provided (**Sample well: DO NOT USE MORE THAN 5 µl OF BLOOD**).
- 2) Add three-four drops (Approximately 140µl) of chase buffer into buffer well.
Note: Only use the buffer provided with Immunospec Malaria kits.
Warning: Too much chase buffer will flood assay making most results uninterpretable.
- 3) Read the test result in 15-20 minutes.
- 4) The control line region will turn from faint blue to red upon successful performance of assay.

INTERPRETATION OF RESULTS



Note: In a valid assay, the control line will turn from faint blue to red.

1) *P. falciparum* Positive reaction

The presence of three-color bands (three bands in all C, 1 and 2 areas) indicates a positive result for *P. falciparum*. The pLDH present in the sample reacts with the pan anti-pLDH conjugate and moves through the test strip where the pLDH is captured by both pan specific anti-pLDH and *P. falciparum*-specific anti-pLDH antibodies.

2) *P. vivax* or other *Plasmodium* sp. Positive reaction

The presence of two color bands (one band in C area and another band in 1 area) indicates a positive result for *P. vivax*, *P. malariae*, or *P. ovale*. The pLDH present in the sample reacts with the pan anti-pLDH conjugate and moves through the test strip where the pLDH is captured by pan specific anti-pLDH.

3) Negative reaction

The presence of only one band (which turns from faint blue to red) in C area within the result window indicates a negative result.

4) Invalid

The test is invalid if the line in C area does not appear. If this occurs, the test should be repeated using a new strip.

Performance Characteristics

Limited sensitivity and specificity have been performed in India. More control and expanded evaluation for Malaria p-LDH antigen detection rapid kit is being planned.

Limited number of confirmed positive (*P.f.*, *P.v.*, *P.m.*, and *P.o.* infected) samples have been tested. In addition, the analytical sensitivity of the test has been determined using purified *P. falciparum* LDH protein. The analytical sensitivity has been found to be at the level of <1 ng/ml.

PRECISION

Within-run and between-run precisions have been determined by the testing 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%.

LIMITATIONS

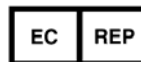
- For Export Use Only
- This test will only indicate the presence of pLDH in whole blood and should not be used as the sole criterion for the diagnosis of malaria (as

with all diagnostic tests, all results must be considered with other clinical information available to the clinicians).

- If the result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. A negative result does not preclude the possibility of malaria.
- Do not use samples containing any glycerol or other viscous materials. This will compromise the sensitivity of the assay dramatically.
- Do not use inadequately anti-coagulated blood or old stored bloods that contain clots and are viscous. Free flowing blood must be used.
- The test is limited to the detection of antigen to Malaria *Plasmodium* sp.
- Although the test is very accurate in detecting pLDH, 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.
- In some cases with very low *P.f.* counts, it is possible only the pan-malaria reactive line 1 will be more visible/clear (line 1) because of high affinity of the pan-malaria antibody combination. Therefore *P.f.* interpretation must be taken in combination with microscopy or require confirmation by a separate test.

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