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PF Malaria Rapid test

Detection of pf Malaria LDH antigen in human blood

REF

Catalog No.R6-537

IVD

For in vitro diagnostic use only
For export use only

Intended Use

The Immunospec Malaria Rapid Test for plasmodium infection is a rapid immunochromatographic strip assay for the qualitative detection of plasmodium lactate dehydrogenase (pLDH) in human blood as an aid in the diagnosis of Malaria infection. This test is intended for in-vitro diagnostic use only.

Summary and Explanation

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). Extreme exhaustion and a sudden febrile illness, sweats, shaking, chills, and anemia characterize the disease. WHO estimates that that 40% of the world's population is at risk of malaria, a disease that was once thought to be under control, but is now resurgent in many developing countries, causing >300 million acute illnesses and upwards of one million deaths annually. Diagnosis of malaria (2-6) has traditionally been done by microscopic examination of blood. Microscopy, however, this 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. 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.

Principle

The Malaria p-LDH antigen test contains a membrane strip, which is pre-coated with a pf specific monoclonal antibody. the test line (T). A conjugate pad is dispensed with monoclonal antibody, which is pan specific to the lactate dehydrogenase of *Plasmodium* species. Once the blood sample is applied, the blood and the anti-pan LDH antibody gold mix migrate upward on the membrane. Reaction occurs with the test line with *P.f* positive samples which generates a red line. The PF Malaria plasmodium pf-LDH Test is designed for specific diagnosis of *Plasmodium falcifarum*. Presence of a red test (T) 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 always appear. The presence of this red line serves as verification for sufficient sample volume and proper flow and as a control for the reagents.

Kit contents

1. Twenty-five (25) test device, individually pouched or 25 tests in a vial.
2. One vial of Chase Buffer solution.
3. Assay tube

Optional:

- Extra assay Buffer
- Sample Pipette (accurately measures 5µl sample blood)
- Buffer Wells
- Lancet
- Alcohol Swab

Precautions

- For in-vitro diagnostic use only. Do not use after expiration date.
- Handle all sera 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 or vial containing the test strip 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 (or sooner for high humidity areas) after removal from the pouch or vial to prevent exposure to humidity.

Specimen Collection and Storage

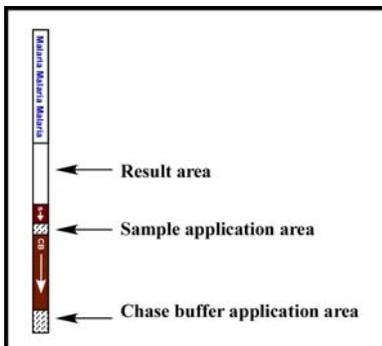
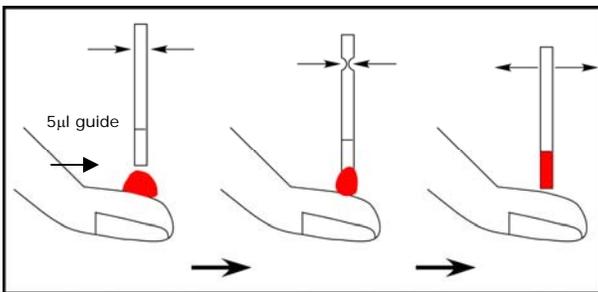
[Collection by venipuncture]

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).
3. If samples are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.

[Collection using a lancet]

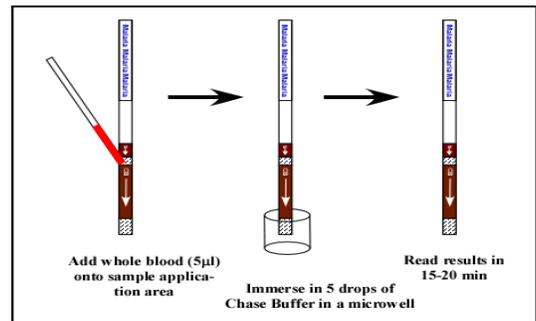
1. Clean the area to be lanced with an alcohol swab.
2. Squeeze the end of the fingertip and pierce with a sterile lancet provided.
3. Wipe away the first drop of blood with sterile gauze or cotton.
4. Using the dropper provided, while gently squeezing the tube, immerse the open end of the tube into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

- 1) Gently squeeze the tube
- 2) Immerse open end in blood
- 3) Gently release to draw blood

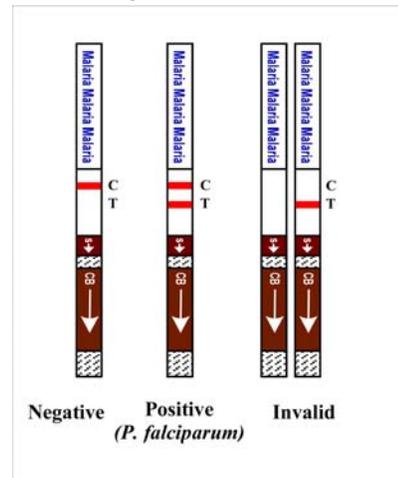


Test Procedure

- 1) Take out one dipstick and one assay tube
- 2) Place the dipstick into the well.
- 3) Add 5 µl of whole blood into sample application area of dipstick using a single channel micropipette or a sample pipette provided. It is advised to gently hold the dipstick at the handling area (the "P.f Malaria" tape), lift from sample well far enough to allow for easy access to sample addition portion of dipstick, then place dipstick back in sample well. **(sample area: DO NOT USE MORE THAN 5 µl OF BLOOD)**.
- 4) Separately add five drops (Approximately 250µl) of chase buffer into buffer well, being sure that the chase buffer drops do not fall directly on the membrane or sample application areas of dipstick.
- 5) Start timer.
- 6) **The control line region of dipstick will turn from a faint blue to red.**
- 7) Read the test result in 15-20 minutes.



Interpretation of the Test



Note: Control line will turn from faint blue to red.

1) *P. falciparum* Positive reaction

The presence of two bands (one band in C area and another band in T area) indicates a positive result for *P. falciparum*. The pf LDH present in the sample reacts with the pan anti-pLDH conjugate and move through the test strip where the pLDH is captured by both *P.*

falciparum-specific anti-pLDH and pan specific anti-pLDH.

3) Negative reaction

The presence of only one red band 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.

NOTE: ONLY READ CLEAR LINES AS POSITIVES. DO NOT READ ANY SHADOW LIKE, OR DIFFICULT TO READ LINES AS POSITIVES.

- Most blood samples clear within 15 minutes. However, with some fresh and also with old blood samples, clearance may take additional 15 minutes. In these situations, allow additional 15 minutes for final reading.
- 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.

Performance Characteristics

The PF **Malaria** pLDH antigen detection rapid kit has been tested with a limited number of confirmed positive (*P.f* and *P.v* infected) and negative clinical samples. 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.

Sensitivity and specificity of Malaria For detection plasmodium LDH

Malaria	Microscopy		
	Positive	Negative	
Positive	30	0	30
Negative	0	70	70
	30	70	100

Sensitivity 100% Specificity 100%

Limited sensitivity and specificity analysis were performed in India. Negative samples included samples from patients presented with fever.

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%.

Limitation

- 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.

References

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