Leishmania IgG/IgM Rapid Test Strip
Package Insert

Cat: ILE-421 **Specimens:** Whole Blood/Serum/Plasma

Version: 02 **Effective Date:** 2024-07

For professional *in vitro* diagnostic use only.

INTENDED USE

The Leishmania IgG/IgM Rapid Test is a lateral flow immunoassay for the qualitative detection of antibodies including IgG and IgM to the RK-39 of the subspecies of the *Leishmania donovani (L. donovani)*, the *Visceral leishmaniasis* causative protozoans in human serum or plasma. This test is intended to be used as a screening test and as an aid in the diagnosis of the disease of *Visceral leishmaniasis.* Any reactive specimen with the Leishmania IgG/IgM Rapid Test must be confirmed with alternative testing method(s).

SUMMARY

Visceral leishmaniasis, or Kala-azar, is a disseminated infection caused by several subspecies of the *L. donovani*. The disease is estimated by the World Health Organization (WHO) to affect approximately 12 million people in 88 countries[1]. It is transmitted to humans by bites of the *Phlebotomus* sandflies, which acquire infection from feeding on infected animals. Though it is a disease for poor countries, in Southern Europe, it has become the leading opportunistic infection in AIDS patients[2-3].

Identification of *L. donovani* organism from the blood, bone marrow, liver, lymph nodes or the spleen provides a definite means of diagnosis. However, these test methods are limited by the sampling method and the special instrument requirement. Serological detection of anti-*L. donovani* Ab is found to be an excellent marker for the infection of *Visceral leishmaniasis*. Tests used in clinic include: ELISA, fluorescent antibody and direct agglutination tests[4-5]. Recently, utilization of *L. donovani* specific protein in the test has improved the sensitivity and specificity dramatically[6-7].

The Leishmania IgG/IgM Rapid Test is a recombinant protein based serological test, which detects antibodies including IgG, IgM and IgA to the *L. Donovani*. This test provides a reliable result within 10 minutes without any instrumentation requirements.

PRINCIPLE

The Leishmania IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant *L. donovani* specific antigen RK-39 conjugated with colloid gold (Leishmania conjugates), 2) a nitrocellulose membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with recombined antigen RK-39 and the C band is pre-coated with chicken anti-protein A.

When an adequate volume of test specimen is dispensed onto the sample pad of the test strip, the specimen migrates by capillary action across the strip. Anti- *L. donovani* Ab if present in the specimen will bind to the RK-39 antigen conjugates. The immunocomplex is then captured on the membrane by the pre-coated recombined antigen RK-39, forming a burgundy colored T band, indicating a *L. donovani* Ab positive test result.

Absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of antibody- chicken anti-protein A -gold conjugate regardless of the color development on the C band. Otherwise, the test result is invalid and the specimen must be retested with another strip.

KIT COMPONENTS

Individually packed test strips	Each strip contains a strip with colored conjugates and reactive reagents pre-spreaded at the corresponding regions
Droppers	For adding specimens use
Buffer	Phosphate buffered saline and preservative
Package insert	For operation instruction

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow the standard procedures for proper disposal of specimens.
- Humidity and temperature can adversely affect results.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at room temperature or refrigerated (2-30°C). The test strip is stable through the expiration date printed on the sealed pouch. The test strip must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

- The Leishmania IgG/IgM Rapid Test strip (Whole Blood/Serum/Plasma) can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.
- To collect Fingerstick Whole Blood specimens:
  Wash the patient's hand then allow to dry. Massage the hand without touching the puncture. Puncture the skin with a sterile lancet. Wipe away the first sign of blood. Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site. Add the Fingerstick Whole Blood specimen to the test strip by using a capillary tube or hanging drops.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.
- Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long-term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

MATERIALS

Materials Provided

- Test strips
- Buffer
- Droppers
- Package insert

Materials Required But Not Provided

- Specimen collection containers
- Centrifuge
- Lancets (for fingerstick whole blood only)
- Timer
- Heparinized capillary tubes and dispensing bulb (for fingerstick whole blood only)

DIRECTIONS FOR USE

1. Bring the pouch to room temperature before opening it. Remove the test device from the sealed pouch and use it as soon as possible.
2. Place the test strip on a clean and level surface.
   Hold the dropper vertically and transfer 2 drops of sample (or approximately 50 µl) to the sample pad of the test strip, then add 1 drop of buffer (or approximately 40 µl) and start the timer.
3. Wait for the red line(s) to appear. The result should be read at 15 minutes. Do not interpret the result after 30 minutes.

INTERPRETATION OF RESULTS

POSITIVE RESULT:

IgG Positive:* The colored line in the control line region (C) appears and a colored line appears in test line region 1 (T1). The result is positive for Leishmania specific-IgG antibodies.

IgM Positive:* The colored line in the control line region (C) appears and a colored line appears in test line region 2 (T2). The result is positive for Leishmania specific-IgM.

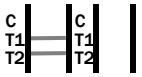
IgG and IgM Positive:* The colored line in the control line region (C) appears and two colored lines should appear in test line regions 1 and 2 (T1 and T2). The color intensities of the lines do not have to match. The result is positive for IgG & IgM antibodies.

***NOTE:** The intensity of the color in the test line region(s) (T1 and/or T2) will vary depending on the concentration of Leishmania antibodies in the specimen. Therefore, any shade of color in the test line region(s) (T1 and/or T2) should be considered positive.

NEGATIVE RESULT:

The colored line in the control line region (C) appears. No line appears in test line regions 1 or 2 (T1 or T2).

INVALID RESULT:



Control line (C) falls to appear. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test strip. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

Using individual Leishmania IgG/IgM Rapid Test strips as described in the Assay Procedure above, run 1 Positive Control and 1 Negative Control (provided upon request) under the following circumstances to monitor test performance:

1. A new operator uses the kit, prior to performing testing of specimens.
2. A new test kit is used.
3. A new shipment of kits is used.
4. The temperature used during storage of the kit falls outside of 2°C-30°C.
5. The temperature of the test area falls outside of 15°C-30°C.

LIMITATIONS OF THE TEST

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of antibodies to the *L. donovani* in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Leishmania Ab Combo Rapid Test is limited to the qualitative detection of antibodies to L. donovani in human serum or plasma. The intensity of the test band does not linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable anti-L. donovani antibodies. However, a negative test result does not preclude the possibility of exposure to Visceral leishmaniasis causative species of the L. donovani
4. A negative result can occur if the quantity of the L. donovani antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

EXPECTED VALUES

The Leishmania IgG/IgM Rapid Test has been compared with a leading commercial EIA test, demonstrating an overall accuracy of 98.9%.

PERFORMANCE CHARACTERISTICS

Clinical Performance

A total of 267 patient samples from susceptible subjects were tested by the Leishmania IgG/IgM Rapid Test and by a commercial *L. donovani* IgG/IgM ELISA kit. Comparison for all subjects is showed in the following table.

	Leishmania IgG/IgM Rapid Test		
EIA	**Positive**	**Negative**	**Total**
Positive	52	2	54
Negative	1	212	213
Total	53	214	267

Relative Sensitivity: 96.3 %, Relative Specificity: 99.5%, Overall Agreement: 98.9%

PRECISION

Intra Assay

Within-run precision has been determined by using 10 replicates of three specimens containing negative, low positive and high positive samples. The negative and positive values were correctly identified >99% of the time.

Inter Assay

Between-run precision has been determined by using the three specimens of negative, low positive and high positive of 10 independent assays and with three different lots of the Leishmania IgG/IgM Rapid Test. The negative and positive values were correctly identified >99% of the time.

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