

# Leishmania IgG/IgM Antibody Rapid Test Cassette (Serum / Plasma)

Cat no. LEISH 471

## INTENDED USE

The Leishmania IgG/IgM Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of IgG and IgM to the subspecies of the *Leishmania donovani* (*L. donovani*), the *Visceral leishmaniasis* causative protozoans, in human serum or plasma. It 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 AND EXPLANATION OF THE TEST

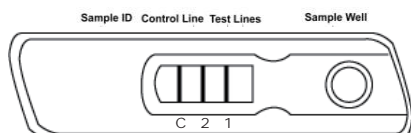
*Visceral leishmaniasis*, or Kala-azar, is a disseminated infection caused by several subspecies of the *L. donovani*. The disease is estimated by the World Health Organization (WHO) to affect approximately 12 million people in 88 countries<sup>1</sup>. It is transmitted to humans by bites of the *Phlebotomus*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

Identification of *L. donovani* organism from the blood, bone marrow, liver, lymph nodes or the spleen provides a definite mean of diagnosis. Serological detection of anti-*L. donovani* IgM is found to be an excellent marker for the acute *Visceral leishmaniasis*. Tests used in clinic are included ELISA, fluorescent antibody or direct agglutination tests<sup>4,5</sup>. Recently, utilization of *L. donovani* specific protein in the test has improved the sensitivity and specificity dramatically<sup>6-7</sup>.

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

## TEST 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* antigen conjugated with colloidal gold (Leishmania conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing two test bands (1 and 2 bands) and a control band (C band). The 1 band is pre-coated with monoclonal anti-human IgM for the detection of anti-*L. donovani* IgM, 2 band is pre-coated with reagents for the detection of anti-*L. donovani* IgG, and the C band is pre-coated with goat anti rabbit IgG.



When an adequate volume of test specimen is dispensed into the sample well of the cassette, the specimen migrates by capillary action across the cassette. The *L. donovani* IgM if present in the specimen will bind to the Leishmania conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM antibody, forming a burgundy colored 1 band, indicating a *L. donovani* IgM positive test result.

The *L. donovani* IgG if present in the specimen will bind to the Leishmania conjugates. The immunocomplex is then captured by the pre-coated reagents on the membrane, forming a burgundy colored 2 band, indicating a *L. donovani* IgG positive test result.

Absence of any T bands (1 and 2) suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat anti rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on any of the T bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

## REAGENTS AND MATERIALS PROVIDED

- Each sealed in a foil pouch with three items inside:
  - One cassette device.
  - One plastic dropper.
  - One desiccant.
- One package insert (instruction for use).

## MATERIALS REQUIRED BUT NOT PROVIDED

- Clock or Timer

## WARNINGS AND PRECAUTIONS

### For in Vitro Diagnostic Use

- This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
- Do not open the sealed pouch, unless ready to conduct the assay.
- Do not use expired devices.
- Bring all reagents to room temperature (15°C-30°C) before use.
- Do not use the components in any other type of test kit as a substitute for the components in this kit.
- Do not use hemolyzed blood specimen for testing.
- Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
- Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Dispose of all specimens and materials used to perform the test as biohazardous waste.
- The testing results should be read within 10 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 10 minutes may give erroneous results.
- Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.

## REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test devices unopened at 2°C-30°C. If stored at 2°C-8°C, ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 30°C.

## SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

### Plasma

- Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively in Vacutainer®) by venipuncture.
- Separate the plasma by centrifugation.
- Carefully withdraw the plasma into new pre-labeled tube.

### Serum

- Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by venipuncture.
- Allow the blood to clot.
- Separate the serum by centrifugation.
- Carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2°C-8°C if not tested immediately.

Store specimens at 2°C-8°C up to 5 days. The specimens should be frozen at -20°C for longer storage.

Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature slowly and mix gently. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Do not use samples demonstrating gross lipemia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

## ASSAY PROCEDURE

- Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.
- When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.
- Be sure to label the device with specimen's ID number.
- Fill the pipette dropper with the specimen. Holding the dropper vertically, dispense 2-3 drops (about 60-90 µl) of specimen into the sample well making sure that there are no air bubbles.



**Note:** Add 1 drop of Saline or Phosphate-Saline buffer (common buffers used in clinic, not provided in the kit) into the sample well if flow migration is not observed within 30 seconds in the result window, which could occur with a highly viscous specimen

- Set up timer.
- Results can be read in 10 minutes. Positive results can be visible in as short as 1 minute.

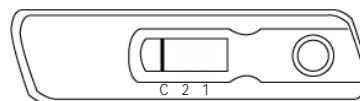
**Don't read result after 10 minutes. To avoid confusion, discard the test device after interpreting the result.**

## QUALITY CONTROL

Although the testing device contains an internal quality control (pink colored band in the control region), good laboratory practice recommends the daily use of an outside control to ensure proper testing device performance. Quality control samples should be tested according to the standard quality control requirements established by your laboratory.

## INTERPRETATION OF ASSAY RESULT

- NEGATIVE RESULT:** If only the C band is present, the absence of any burgundy color in the both T bands (1 and 2) indicates that no anti-*L. donovani* antibody is detected in the specimen. The result is negative.



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### 2. POSITIVE RESULT:

2.1 In addition to the presence of C band, if only 1 band is developed, the test indicates for the presence of IgM anti-*L. donovani* in the specimen. The result is positive.



2.2 In addition to the presence of C band, if only 2 band is developed, the test indicates for the presence of IgG anti-*L. donovani* in the specimen. The result is positive.

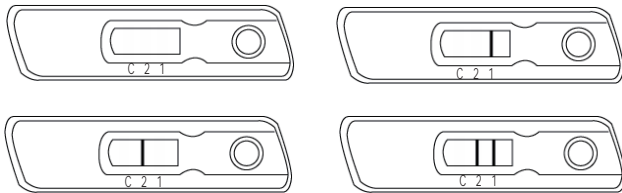


2.3 In addition to the presence of C band, both 1 and 2 bands are developed, the test indicates for the presence of both IgG and IgM anti-*L. donovani* in the specimen. The result is also positive.



Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. **INVALID:** If no C band is developed, the assay is invalid regardless of any burgundy color in the T bands as indicated below. Repeat the assay with a new device.



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.

### REFERENCES

1. WHO. Control of the Leishmaniasis. World Health Organization. Technical Report Series 1990. No. 793.
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3. Molina R, Gradoni L, Alvar J. HIV and the transmission of Leishmania. Ann Trop Med Parasitol. 2003 ;97 Suppl 1:29-45.
4. Allain DS, Kagan IG. A direct agglutination test for leishmaniasis. Am J Trop Med Hyg. 1975 ;24(2):232-6.
5. Badaro R, Reed SG, Carvalho EM. Immunofluorescent antibody test in American visceral leishmaniasis: sensitivity and specificity of different morphological Am J Trop Med Hyg. 1983;32(3):480-4.
6. Maalej IA, Chenik M, Louzir H, Ben Salah A, et al. Comparative evaluation of ELISAs based on ten recombinant or purified Leishmania antigens for the serodiagnosis of Mediterranean visceral leishmaniasis. Am J Trop Med Hyg. 2003 68(3):312-20.
7. Burns JM Jr, Shreffler WG, Benson DR, Ghalib HW, Badaro R, Reed SG. Molecular characterization of a kinesin-related antigen of Leishmania chagasi that detects specific antibody in African and American visceral leishmaniasis. Proc Natl Acad Sci U S A. 1993 Jan 15;90(2):775-9.

### MANUFACTURER

ATLAS LINK TECHNOLOGY CO., LTD  
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WEB: <https://www.invitro-test.com>

### PERFORMANCE CHARACTERISTICS

#### 1. Clinical Performance For IgM Test

34 specimens from fresh infected patients mixed with 300 specimens derived from non-infected individuals were tested by the Leishmania IgG/IgM Rapid Test. The test result is showed in the following table.

Clinic status	Leishmania IgG/IgM Rapid Test		Total
	Positive	Negative	
Fresh infected	31	3	34
Non-infected	2	298	300
<b>Total</b>	<b>33</b>	<b>302</b>	<b>334</b>

Relative Sensitivity: 91% , Relative Specificity: 99.6%, Overall Agreement: 98.5%

#### 2. Clinical Performance For IgG Test

A total of 314 patient samples from susceptible subjects were tested by the Leishmania IgG/IgM Rapid Test and by a commercial *L. donovani* IgG EIA kit. Comparison for all subjects is showed in the following table.

IgG EIA	Leishmania IgG/IgM Rapid Test		Total
	Positive	Negative	
Positive	13	1	14
Negative	2	298	300
<b>Total</b>	<b>15</b>	<b>299</b>	<b>314</b>

Relative Sensitivity: 92.9%, Relative Specificity: 99.3%, Overall Agreement: 99.0%

### LIMITATIONS OF 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 IgG/IgM 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 have 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.

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