



#### INTENDED USE

The **Reliable one step** Leishmania IgG/IgM Combo Rapid Test device 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, plasma or whole blood. 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 **Reliable one step** Leishmania IgG/IgM Combo Rapid Test device must be confirmed with alternative testing method(s).

#### SUMMARY AND EXPLANATION OF THE TEST

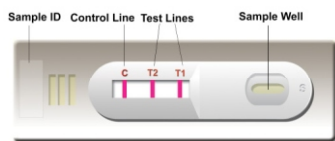
*Visceral leishmaniasis*, or Kala-azar, is a disseminated infection caused by several subspecies of the *L. donovani*. The disease is estimated by the World Health Organization (WHO) to affect approximately 12 million people in 88 countries<sup>1</sup>. It is transmitted to humans by bites of the *Phlebotomus* 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 **Reliable one step** Leishmania IgG/IgM Combo Rapid Test device 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 15 minutes without any instruments.

#### TEST PRINCIPLE

The **Reliable one step** Leishmania IgG/IgM Combo Rapid Test device 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 (T1 and T2 bands) and a control band (C band). The T1 band is pre-coated with monoclonal anti-human IgM for the detection of anti-*L. donovani* IgM, T2 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 T1 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 T2 band, indicating a *L. donovani* IgG positive test result.

Absence of any T bands (T1 and T2) 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 kit contains 50 test devices, each sealed in a foil pouch with three items inside.
  - One cassette device.
  - One plastic dropper.
  - One desiccant.
- Sample diluent (2 bottle, 5 mL)
- One package insert (instruction for use)

#### 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.
- Handle the Negative and Positive Control in the same manner as patient specimens.
- The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 15 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 -3°C. The positive and negative controls should be kept at 2°C -8°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 veinpuncture.
- 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 veinpuncture.
- 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.

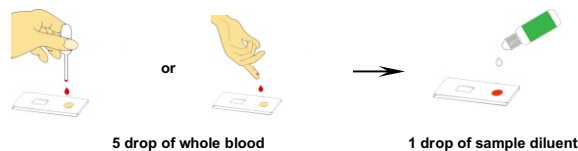
##### Blood

Drops of whole blood can be obtained by either finger tip puncture or veinpuncture. Do not use any hemolyzed blood for testing.

Whole blood specimens should be stored in refrigeration (2°C-8°C) if not tested immediately. The specimens must be tested within 24 hours of collection.

#### 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.
- For whole blood test**  
Apply 5 drop of whole blood (about 40-50 µL) into the sample well.  
Then add 1 drop (about 35-50 µL) of Sample Diluent immediately.

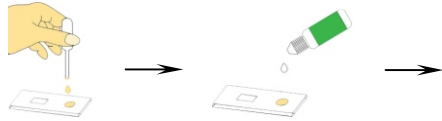


##### For serum or plasma test

Fill the pipette dropper with the specimen.

Holding the dropper vertically, dispense 4 drops (about 30-45 µL) of specimen into the sample well making sure that there are no air bubbles.

Then add 1 drop (about 35-50 µL) of Sample Diluent immediately.



4 drop of specimen      1 drop of sample diluent      15 minutes

Step 5: Set up timer.

Step 6: Results can be read in 15 minutes. Positive results can be visible in as short as 1 minute.

**Don't read result after 15 minutes. To avoid confusion, discard the test device after interpreting the result.**

**QUALITY CONTROL**

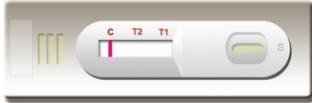
Using individual *Reliable one step* Leishmania IgG/IgM Combo Rapid Test device as described in the Assay Procedure above, run 1 Positive Control and 1 negative Control (provided upon request) under the following circumstances to monitor test performance:

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.

Expected results are as follows:

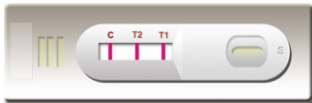
**Negative Control**

Only the C band shows color development, the two T bands (T1 and T2) show no color development.



**Positive Control**

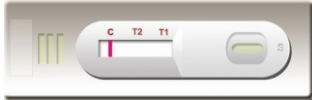
The C band and two T bands (T1 and T2) show color development.



The appearance of any burgundy color in the T bands, regardless of intensity, must be considered as presence of the band.

**INTERPRETATION OF ASSAY RESULT**

**1. NEGATIVE RESULT :** If only the C band is present, the absence of any burgundy color in both T bands (T1 and T2) indicates that no anti-*L. donovani* antibody is detected in the specimen. The result is negative.



**2. POSITIVE RESULT**

**2.1** In addition to the presence of C band, if only T1 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 T2 band is developed, the test indicates for the presence of IgG anti-*L. donovani* in the specimen. The result is positive.

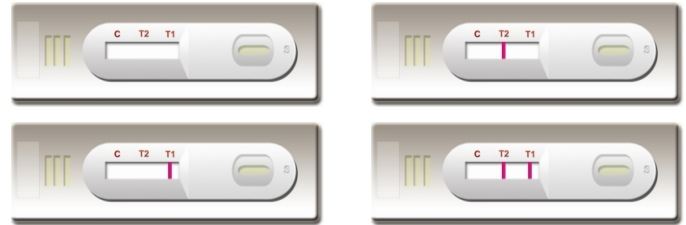


**2.3** In addition to the presence of C band, both T1 and T2 bands are developed, the test indicates for the presence of both IgG and IgM anti-*L. Donovani* in the specimen. The result is also positive.



*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.



**PERFORMANCE CHARACTERISTICS**

**1. Clinical Performance For IgM Test**

A total of 234 samples from susceptible subjects were tested by the *Reliable one step* Leishmania IgG/IgM Combo Rapid Test and by a commercial *L. donovani* IgM EIA. Comparison for all the subjects is showed in the following table.

Reliable one step Leishmania IgG/IgM Combo Rapid Test			
IgM EIA	Positive	Negative	Total
Positive	31	3	34
Negative	2	199	200
<b>Total</b>	<b>33</b>	<b>202</b>	<b>234</b>

Relative Sensitivity: 91.2% , Relative Specificity: 99.5%, Overall Agreement: 98.3%

**2. Clinical Performance For IgG Test**

A total of 214 samples from susceptible subjects were tested by the *Reliable one step* Leishmania IgG/IgM Combo Rapid Test and by a commercial *L. donovani* IgG EIA kit. Comparison for all subjects is showed in the following table.

Reliable one step Leishmania IgG/IgM Combo Rapid Test			
IgG EIA	Positive	Negative	Total
Positive	13	1	14
Negative	2	198	200
<b>Total</b>	<b>15</b>	<b>199</b>	<b>214</b>

Relative Sensitivity: 92.9% , Relative Specificity: 99.0%, Overall Agreement: 98.6%

**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, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *Reliable one step* Leishmania IgG/IgM Combo Rapid Test device is limited to the qualitative detection of antibodies to *L. donovani* in human serum, plasma or whole blood. 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.
5. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical finding.

**REFERENCES**

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