BIOGENIX
INC. PVT. LTD.

Leishmania IgG/IgM Rapid Test Cassette

(Immunochromatography)

Product Name

Leishmania IgG/IgM Rapid Test Cassette (Immunochromatography)

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).

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 countries1. 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.

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 4-5. Recently, utilization of *L. donovani* specific protein in the test has improved the sensitivity and specificity dramatically.

TheLeishmania 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

TheLeishmania 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 colloid 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 IgG for the detection of anti-*L. donovani* IgG, T2 band is pre-coated with reagents for the detection of anti-*L. donovani* IgM, and the C band is pre-coated with goat anti rabbit IgM.

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* IgG 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 IgG antibody, forming a burgundy colored T1 band, indicating a *L. donovani* IgG positive test result.

The *L. donovani* IgM 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* IgM 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.

Main Components

Sample pad, colloidal gold marked pad, nitrocellulose membrane, absorbent paper and PVC board.

Storage and Expiry

Store as packaged in the sealed pouch at 2-30°C, avoid hot and sunshine, dry place, valid for 24 months. DO NOT FREEZE. Some protective measures should be taken in hot summer and cold winter to avoid high temperature or freeze-thaw.

Sample Requirement

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

Plasma

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

Serum

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
2. Allow the blood to clot.
3. Separate the serum by centrifugation.
4. 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.

Test Procedure

Instructions must be read entirely before taking the test. Allow the test device controls to equilibrate to room temperature for 30 minutes (20℃-30℃) prior to testing. Do not open the inner packaging until ready, it must be used in one hour if opened (Humidity: 20%~90%, Temp: 10℃-50℃)

1. Take off the outer packing, put the cassette onto the desk with the sample window up.

2. Drop 1 drops of serum or plasma (20μl-30μl) vertically into the sample hole (S) of cassette well, making sure that there are no air bubbles. Add about 2 drops of (80μl-100μl) sample buffer into the sample hole (S) of cassette immediately.

3. Observe the test results immediately within 15 minutes.

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

Result Judgment

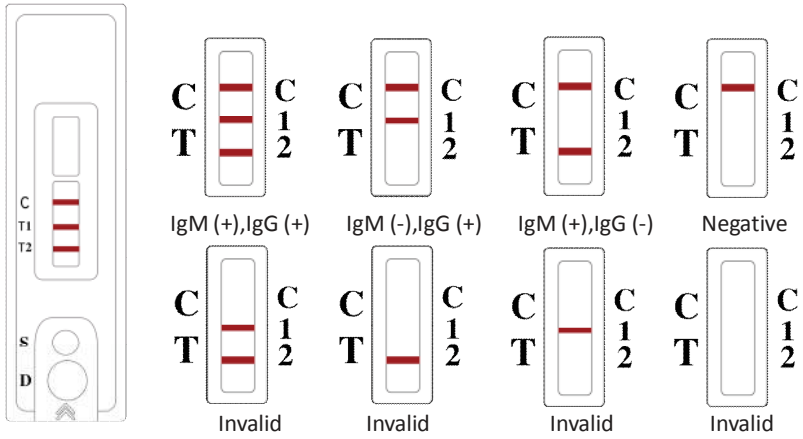
POSITIVE: Two distinct red lines appear. One line should be in the control region (C) and another line should be in the T1 test region (T1), indicating the presence of IgG anti-*L. donovani* in the specimen.

POSITIVE: Two distinct red lines appear. One line should be in the control region (C) and another line should be in the T2 test region (T2), indicating the presence of IgM anti-*L. donovani* in the specimen.

POSITIVE: Three distinct red lines appear in the control region (C), the T1 test region (T1) and the T2 test region (T2), indicating the presence of both IgG and IgM anti-*L. donovani* in the specimen .

NEGATIVE: One red line appears in the control region(C). No apparent red or pink line appears in the test region (T1 and T2).

INVALID: No red bands appear or control line fails to appear, indicating that the operator error or reagent failure.



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 theLeishmania IgG/IgM Rapid Test. The test result is showed in the following table.

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

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 theLeishmania IgG/IgM Rapid Test and by a commercial *L. donovani* IgG EIA kit. Comparison for all subjects is showed in the following table.

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

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

Limitation

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. TheLeishmania 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.
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.

Precaution

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not open the sealed pouch, unless ready to conduct the assay.
3. Do not use expired devices.
4. Bring all reagents to room temperature (15°C-30°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolized blood specimen for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as biohazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. 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.
13. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.

Manufacturer

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