

LEISHMANIA IgG/IgM CARD

For in Vitro diagnostic use only

Immunochromatographic test card for qualitative detection of IgG/IgM antibody to Leishmania in human serum, plasma and whole blood

I. INTENDED USE

The Leishmania IgG/IgM Card 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 Leishmania IgG/IgM Card Test must be confirmed with alternative testing method(s).

II. 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 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 patients²⁻³.

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 tests⁴⁻⁵. Recently, utilization of *L. donovani* specific protein in the test has improved the sensitivity and specificity dramatically⁶⁻⁷.

The Leishmania IgG/IgM Card 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.

III. TEST PRINCIPLE

The Leishmania IgG/IgM Card is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant *L. donovani* specific antigen conjugated with colloid gold (Leishmania conjugates) and rabbit IgG-gold 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 un-conjugated *L. donovani* antigen, and the C band is pre-coated with goat anti-rabbit IgG antibody.

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

Absence of 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 goat anti-rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on the T band. Otherwise, the test result is invalid and the specimen must be retested with another device.

IV. REAGENTS AND MATERIALS PROVIDED

- Each kit contains 10 test devices, each in a foil pouch with following items inside:
 - One cassette device
 - One dessicant.
- Plastic droppers (10 items)
- Sample diluent (1 bottle, 1.0 ml)
- One package insert.

V. MATERIALS REQUIRED BUT NOT PROVIDE

- Specimen collection containers
- Clock or timer
- Centrifuge
- Lancing device and Heparinized capillary tubes and dispensing bulb for fingerstick whole blood test only

VI. PRECAUTIONS

1. For professional and in vitro diagnostic use.
2. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
3. Do not open the sealed pouch, unless ready to conduct the assay.
4. Do not use expired devices.
5. Bring all reagents to room temperature (15°C-30°C) before use.
6. Do not use the components in any other type of test kit as a substitute for the components in this kit.
7. Do not use hemolyzed blood specimen for testing.
8. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
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. The testing results should be read within 15 minutes after a specimen is applied to the sample well of the device. Do not read result after 30 minutes. May give erroneous results.
12. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.



VII. REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test device 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.

VIII. SPECIMEN COLLECTION AND HANDLING

The Leishmania IgG/IgM Card can be performed using whole blood (from venepuncture or fingerstick), serum or plasma. Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

Fingerstick whole blood specimens and whole blood

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 device by using a capillary tube or hanging drops.

Do not use any hemolized 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.

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.

IX. ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed.

Step 2: *When ready to test, open the pouch and remove device. Place the test device on a clean, flat surface.*

Step 3: Be sure to label the device with specimen's ID number.

Step 4: **For whole blood, Serum or Plasma specimens:**

Hold the dropper vertically and transfer **2 drops of specimens** (about 80 µL) into the specimen well (S) of the device, then add **1 drop of Sample Diluent** (about 40 µL) for serum or plasma and **2 drops of Sample Diluent** (about 80 µL) for whole blood.

For Fingerstick Whole Blood specimens:

To use a capillary tube: fill the capillary tube and **transfer approximately 50 µL (or 2 drops) of fingerstick whole blood specimen** to the specimen well (S) of the device test, then add **2 drops of Sample Diluent** (about 80 µL) immediately.

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 30 minutes. To avoid confusion, discard the test device after interpreting the result.

X. INTERPRETATION OF ASSAY RESULT

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



2. **POSITIVE RESULT** 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*; The result is positive.



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*. The result is positive.

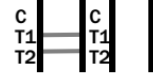


In addition to the presence of C band, both T1 and T2 bands are developed, the test indicates for the presence of both IgG and IgM anti- *L. donovani*. The result is also positive.



**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. 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. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Repeat the assay with a new device. If the problem persists, discontinue using the test kit immediately and contact your local distributor



XI. PERFORMANCE CHARACTERISTICS

Clinical Performances

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

EIA	Leishmania IgG/IgM Card MB		Total
	Positive	Negative	
Positive	31	3	34
Negative	1	199	200
Total	32	202	234

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

XII. LIMITATIONS OF TEST

- 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.
- The Leishmania IgG/IgM Card 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.
- 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*
- 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.
- Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
- The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical finding.

XIII. REFERENCES

- WHO. Control of the Leishmaniasis. World Health Organization. Technical Report Series 1990. No. 793.
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- Allain DS, Kagan IG. A direct agglutination test for leishmaniasis. Am J Trop Med Hyg. 1975 ;24(2):232-6.
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- Burns JM Jr, Shreffler WG, Benson DR, Ghalib HW, Badaro R, Reed SG. Molecular characterization of a kinsin-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.

IVD	In Vitro Diagnostic Medical Device	Temperature limitation	LOT	Batch code (EXXX)	Manufacturer	Keep dry	Non-sterile
Consult Instructions for use	Use by (year/month)	REF	Catalogue number	Do not reuse	Fragile, handle with care	Keep away from heat	

CONTENT (10 tests)

Leishmania Card
Sample diluent
Instruction for use

COD. VQ85200

10 devices
1 x 1.0 mL
1 item

EDMA Code 15 05 10 05 00

