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 whoole blood

I. INTENDED USE

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

II. SUMMARY AND EXPLANATION OF THE TEST

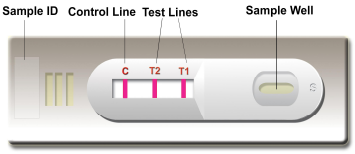
Visceral leishmaniasis, or Kala-azar, is a disseminated infection caused by several subspecies of the *L. donovani*. The disease is estimated by the World Health Organization (WHO) to affect approximately 12 million people in 88 countries[1]. It is transmitted to humans by bites of the *Phlebotomus* sandflies, which acquire infection from feeding on infected animals. Though it is a disease found in 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 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[6-7].

The Leishmania 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 15 minutes without any instruments.

III. TEST PRINCIPLE

The Leishmania Card 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 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.

IV. REAGENTS AND MATERIALS PROVIDED

- Each kit contains 10 test devices, each in a foil pouch with three items inside:
  - One cassette device
  - One plastic dropper
  - One dessicant.
- Sample diluent (1 bottle, 1.6 ml)
- One package insert.

V. MATERIALS REQUIRED BUT NOT PROVIDE

- Clock or timer
- Lancing device for whole blood test.

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 hemolized 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 or sample pad of the device. Read result after 15 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

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 lipermia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

Whole blood

Drops of whole blood can be obtained by either finger tip puncture or veinpuncture. 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.

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.

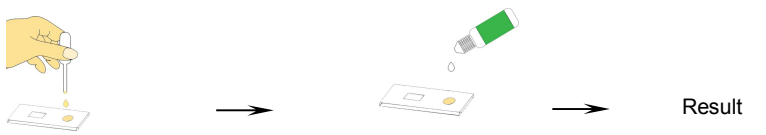
Step 3: Be sure to label the device with specimen's ID number.
Step 4: **For whole blood test**
Apply 1 drop of whole blood (about 40-50 µL) into the sample well. Then add 1 drop (about 35-50 µL) of Sample Diluent immediately.

1 drop of whole blood **1 drop of Sample Diluent**

For serum or plasma test

Fill the pipette dropper with the specimen.

Holding the dropper vertically, dispense 1 drop (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.

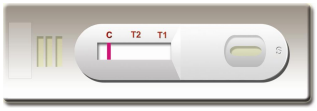


1 drop of sample *1 drop of Sample Diluent* *15 minutes*

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.

X. INTERPRETATION OF ASSAY RESULT

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



2. **POSITIVE**:

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*; 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*. 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*. 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.

XI. PERFORMANCE CHARACTERISTICHS

Clinical Performances for IgM test

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

	Leishmania Card MB		
IgM EIA	**Positive**	**Negative**	**Total**
Positive	31	3	34
Negative	2	199	200
Total	33	202	234

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

Clinical Performances for IgG test

A total of 214 samples from susceptible subjects were tested by the Leishmania Card and by a commercial *L. donovani* IgG EIA kit. Comparison for all subjects is showed in the following table.

	Leishmania Card MB		
IgG EIA	**Positive**	**Negative**	**Total**
Positive	13	1	14
Negative	2	198	200
Total	15	199	214

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

XII. 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 Leishmania 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.
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.

XIII. REFERENCES

1. WHO. Control of the Leishmaniasis. World Health Organization. Technical Report Series 1990. No. 793.
2. Rosenthal E, Marty P. Visceral leishmaniases. Rev Prat. 2004;54(20):2211-6.
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.

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	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)	**COD. VQ85200**
Lehismania Card	10 devices
Sample diluent	1 x 1.6 mL
Instruction for use	1 item

EDMA Code 15 05 10 05 00 CE IVD