Leishmania IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) Package Insert

REF ILEI-402	English

A rapid test for the qualitative detection of IgG and IgM antibodies to Leishmania in human's whole blood, serum or plasma specimen.
For professional in vitro diagnostic use only.

INTENDED USE

The Leishmania IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies to Leishmania in human's whole blood, serum or plasma.

SUMMARY

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 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) 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.

PRINCIPLE

The Leishmania IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) 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 (IgM and IgG bands) and a control band (C band). The IgM band is pre-coated with monoclonal anti-human IgM for the detection of anti-L. donovani IgM, IgG 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.

REAGENTS

The test cassette contains recombinant Leishmania antigen conjugated colloid gold, mouse anti-human IgG and mouse anti-human IgM coated on the membrane.

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.

STORAGE AND STABILITY

The kit can be stored at room temperature or refrigerated (2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

- The Leishmania IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.
- To collect Fingerstick Whole Blood specimens:
  - Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
  - Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
  - 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 by using a capillary tube:
    - Touch the end of the capillary tube to the blood until filled to approximately 40 µL. Avoid air bubbles.
    - Place the bulb onto the top end of the capillary tube, then squeeze the bulb to dispense the whole blood to the specimen well of the test cassette.
  - Add the Fingerstick Whole Blood specimen to the test by using hanging drops:
    - Position the patient's finger so that the drop of blood is just above the specimen well of the test cassette.
    - Allow 1 hanging drop of fingerstick whole blood to fall into the center of the specimen well on the test cassette, or move the patient's finger so that the hanging drop touches the center of the specimen well. Avoid touching the finger directly to the specimen well.
- Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens can be used.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations for transportation of etiologic agents.
- EDTA K2, Heparin sodium, Citrate sodium and Potassium Oxalate can be used as the anticoagulant for collecting the specimen.

MATERIALS

Materials provided

- Test cassettes
- Droppers
- Buffer
- Package insert

Materials required but not provided

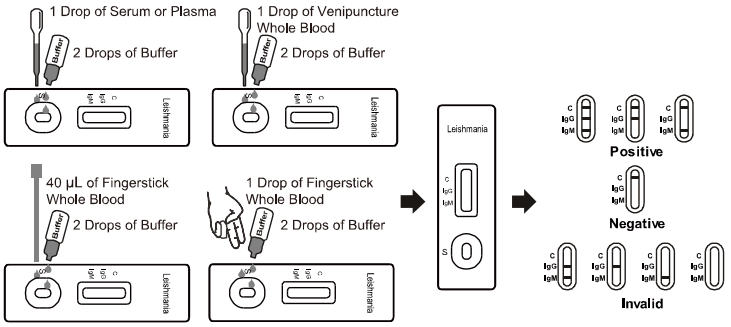
- Specimen collection containers
- Centrifuge
- Timer
- Lancets (for fingerstick whole blood only)
- Heparinized capillary tubes and dispensing bulb (for fingerstick whole blood only)

DIRECTIONS FOR USE

Allow test cassette, specimen and/or controls to equilibrate to room temperature (15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
2. Place the cassette on a clean and level surface.
   For **Serum or Plasma** specimen: Hold the dropper vertically and **transfer 1 drop of serum or plasma** (approximately 40 µL) to the specimen well, then **add 2 drops of buffer** (approximately 80 µL),and start the timer, see illustration below.
   For **Venipuncture Whole Blood** specimen: Hold the dropper vertically and **transfer 1 drop of whole blood** (approximately 40 µL) to the specimen well, then **add 2 drops of buffer** (approximately 80µL), and start the timer. See illustration below.
   For **Fingerstick Whole Blood** specimen:
   - To use a capillary tube: Fill the capillary tube **and transfer approximately 40 µL of fingerstick whole blood specimen** to the specimen well of test cassette, then **add 2 drops of buffer** (approximately 80 µL) and start the timer. See illustration below.
   - To use hanging drops: Allow **1 hanging drop of fingerstick whole blood specimen** (approximately 40 µL) to fall into the specimen well of test cassette, then **add 2 drops of buffer** (approximately 80 µL) and start the timer. See illustration below.
3. Wait for the colored line(s) to appear. **Read the result at 15 minutes**, do not interpret the result after 20 minutes.

Note: It is suggested not to use the buffer, beyond 6 months after opening the vial.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

IgG POSITIVE: Two colored lines appear. One colored line should be in the control region (C) and another colored line should be in the IgG region.
IgM POSITIVE: Two colored lines appear. One colored line should be in the control region (C) and another colored line should be in the IgM region.
IgG and IgM POSITIVE: Three colored lines appear. One colored line should be in the control region (C) and another two colored lines should be in the IgG and IgM regions.
NOTE: The intensity of the color in the test line region (T) will vary depending on the concentration of Leishmania antibodies present in the specimen. Therefore, any shade of red in the test region should be considered positive.
NEGATIVE: One colored line appears in the control region (C). No line appears in the IgG and IgM region.
INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is an internal procedural control. It confirms sufficient specimen volume and correct procedural technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1. The DIRECTIONS FOR USE and the INTERPRETATION OF RESULTS 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 IgG/IgM Rapid Test Cassette 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.
6. The hematocrit level of the whole blood can affect the test results. Hematocrit level needs to be between 25% and 65% for accurate results.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

A total of 269 samples from susceptible subjects were tested by the Leishmania IgG/IgM Rapid Test Cassette and by a commercial L. donovani IgM ELISA. Comparison for all subjects is shown in the following table.

IgM Results

Method		ELISA		Total Results
Leishmania IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	31	4	35
	Negative	3	231	234
Total Results		34	235	269

Relative Sensitivity: 91.2% (95%CI*: 76.3%-98.1%)
Relative Specificity: 98.3% (95%CI*: 95.7%-99.5%)
Accuracy: 97.4% (95%CI*: 94.7%-98.9%) *Confidence Intervals

A total of 314 samples from susceptible subjects were tested by the Leishmania IgG/IgM Rapid Test Cassette and by a commercial L. donovani IgG ELISA. Comparison for all subjects is shown in the following table.

IgG Results

Method		ELISA		Total Results
Leishmania IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	13	6	19
	Negative	1	294	295
Total Results		14	300	314

Relative Sensitivity: 92.9% (95%CI*: 66.1%-99.8%)
Relative Specificity: 98.0% (95%CI*: 95.7%-99.3%)
Accuracy: 97.8% (95%CI*: 95.5%-99.1%) *Confidence Intervals

Precision

Intra-Assay

Within-run precision has been determined by using 20 replicates of five specimens: a negative, a Leishmania IgM low titer positive, a Leishmania IgM high titer positive, a Leishmania IgG low titer positive and a Leishmania IgG high titer positive. The negative, a Leishmania IgM low titer positive, a Leishmania IgM high titer positive, a Leishmania IgG low titer positive and a Leishmania IgG high titer positive values were correctly identified 100% of the time.

Inter-Assay

Between-run precision has been determined by 20 independent assays on the same five specimens: a negative, a Leishmania IgM low titer positive, a Leishmania IgM high titer positive, a Leishmania IgG low titer positive and a Leishmania IgG high titer positive. Three different lots of the Leishmania IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) have been tested by using negative, a Leishmania IgM low titer positive, a Leishmania IgM high titer positive, a Leishmania IgG low titer positive and a Leishmania IgG high titer positive specimens. The specimens were correctly identified 100% of the time.

Cross-reactivity

The Leishmania IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb,anti-Syphilis, anti-HIV, anti-H.Pylori, anti-MONO, anti-CMV IgG, anti-CMV IgM, anti-Rubella IgG, anti-Rubella IgM, anti-Toxo IgG and anti-Toxo IgM positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to Leishmania negative and positive specimens.

Acetaminophen: 20 mg/dL	Caffeine: 20 mg/dL	Acetylsalicylic Acid: 20 mg/dL
Gentisic Acid: 20 mg/dL	Ascorbic Acid: 2 g/dL	Albumin: 2 g/dL
Creatin: 200 mg/dL	Hemoglobin: 1000 mg/dL	Bilirubin: 1g/dL
Oxalic Acid: 60 mg/dL		

None of the substances at the concentration tested interfered in the assay.

BIBLIOGRAPHY

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

Index of Symbols

	Consult instructions for use or consult electronic instructions for use		Contains sufficient for <n> tests	30°C 2°C	Temperature limit
IVD	*In vitro* diagnostic medical device	LOT	Batch code	REF	Catalogue number
EC REP	Authorized representative in the European Community/European Union		Use-by date		Do not re-use
	Do not use if package is damaged and consult instructions for use		Manufacturer		Caution

ACRO BIOTECH, Inc.
4650 Arrow Highway,
Suite D-6 Montclair, CA 91763, U.S.A.
Tel: +1 (909) 541-5085 www.acrobiotech.com

CE

EC REP
MedNet EC-REP GmbH
Borkstrasse 10
48163 Muenster
Germany

Number: 145234703
Revision date: 2023-10-11