QUANTON BIOLIFE SCIENCES

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Leishmania IgG/IgM Ab Rapid Test Kit (Serum/Plasma/Whole blood)

QBL/Lei/RPT_050

The Leishmania IgG/IgM Ab Rapid Test is a rapid chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies to *L. donovani (Leishmania)* in human serum/plasma/whole blood specimens.

For *In-Vitro Diagnostic* Use only

ORDER INFORMATION

Pack Size	REF
01 Test	PLSH 01
05 Tests	PLSH 05
10 Tests	PLSH 10
25 Tests	PLSH 25
50 Tests	PLSH 50

CLINICAL SIGNIFICANCE

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 for 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 definite means of diagnosis. However, these test methods are limited by the sampling method and special instrument requirement. Serological detection of anti-L. donovani Ab is found to be an excellent marker for the infection of Visceral leishmaniasis. Tests used in clinics include: ELISA, fluorescent antibody and direct agglutination tests[4-5]. Recently, utilization of L. donovani specific protein in the test has improved the sensitivity and specificity dramatically[5].

PRINCIPLE

Leishmania IgG/IgM Rapid Test is a lateral flow chromatographic immunoassay. The strip test components consist of: 1) a colored conjugate pad containing recombinant *L. donovani* antigens conjugated with colloidal gold (antigen conjugates) and a control antibody conjugated with colloidal gold, and 2) a nitrocellulose membrane strip containing IgG line (IgG line), IgM line (IgM Line) and a control line (C line). The IgG line is pre-coated with Anti-human IgG antibodies to *L. donovani*, The IgM line is Pre-coated with Anti-human IgM antibodies to *L. donovani* and the C line is pre-coated with a control line antibody. During the assay, when an adequate volume of test specimen is dispensed into the specimen well (S) of the test cassette and a sample diluent is added to the buffer well (B), the specimen migrates by capillary action across the strip held in the cassette. Specific antibody IgG of *L. donovani*, if present in the specimen will bind to the recombinant *L. donovani* colloidal gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated Anti-human IgG antibodies, forming a colored IgG band, indicating a Leishmania IgG antibody positive test result. Alternatively, IgM if present in the specimen will bind to the recombinant *L. donovani* antigen colloidal gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated Anti-human IgM antibodies, forming a colored IgM band, indicating a Leishmania IgM positive test result. The test contains an internal control (C line), which should exhibit a colored line of the immunocomplex of the control antibodies regardless of color development on the test lines. If the C line does not develop, the test result is invalid and the specimen must be retested with another device.

KIT COMPONENTS

• Test Cassettes • Droppers • Buffer • Package Insert

MATERIALS REQUIRED BUT NOT PROVIDED

• Specimen Collection Containers • Centrifuge (For plasma only) • Timer

PRECAUTIONS

1. For professional *in vitro* diagnostic use only. Do not use after the expiration date.
2. Wear protective gloves while handling specimens wash thoroughly afterwards.
3. The device is sensitive to humidity as well as heat. Therefore, take out the device from seal pouch before test.
4. Do not mix reagents from different lot.
5. Dispose all the samples and kits properly as per the instruction after test in accordance in GLP.
6. Follow the testing procedure exactly as mention in the insert.

STORAGE AND STABILITY

1. The kit can be stored at room temperature or refrigerated (2-30°C). The test device must remain in the sealed pouch until use. DO NOT FREEZE.
2. Do not use beyond the expiration date.
3. Do not use the test kit, if the pouch is damaged or seal is broken.

SPECIMEN COLLECTION & PREPARATION

The Leishmania IgG/IgM Ab Rapid Test Cassette (Whole Blood/Serum/Plasma) can be performed using whole blood, serum and plasma specimen.

- **Serum (S):** Collect the whole blood into a collection tube (NOT containing anticoagulants such as heparin, EDTA, and sodium citrate) by vein puncture, leave to settle for 30 minutes for blood coagulation and then centrifuge blood to get serum specimen of supernatant.
- **Plasma (P):** Collect the whole blood into a collection tube (containing anticoagulants such as EDTA K2, Heparin sodium, Citrate sodium and Oxalate potassium) by vein puncture and then centrifuge blood to get plasma specimen.
- **Whole Blood (WB):** Both Fingerstick Whole Blood and Venipuncture Whole Blood can be used.

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 sample well of the test cassette by using a sample dropper. Avoid air bubbles.
- Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. For long term storage, specimens should be kept below -20°C. 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.

DIRECTIONS FOR USE

Allow the test device, specimen and/or buffer to equilibrate at room temperature (15-30°C) before testing.

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it within 1 hour.
2. Place the cassette on a clean and level surface.
3. **For Serum or Plasma specimen**: 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 (5 µL) to the specimen well, then add 2 drops of buffer (approximately 80 µL) and start the timer
4. **For Venipuncture Whole Blood specimen**: Hold the dropper vertically and transfer 1 drop of whole blood (5µL) to the specimen

well, then add 1 drops of buffer (approximately 80 µL) and start the timer

5. **For Fingerstick Whole Blood specimen:** Hold the dropper vertically and transfer 1 drop of whole blood (5 µL) to the specimen well, then add 2 drops of buffer (approximately 80 µL) and start the timer

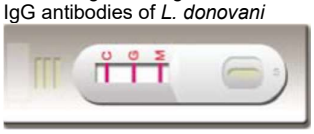
Wait for the colored line(s) to appear. Read results at 15 minutes.
Note: Do not interpret the result after 20 minutes.

INTERPRETATION OF RESULTS

1) **IgG POSITIVE:** Two distinct red lines appear. The control line (c) and IgG (G) line are visible on the test cassette. This indicates the presence of IgG antibodies of *L. donovani* in specimen. The result is positive

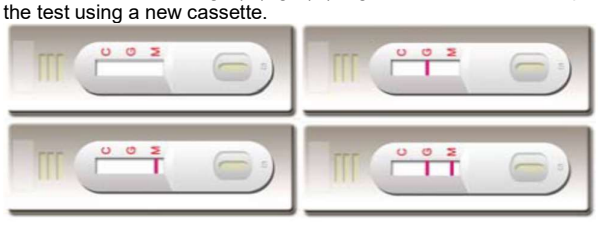
2) **2) IgM POSITIVE:** Two distinct red lines appear. The control line (c) and IgM (M) line are visible on the test cassette. This indicates the presence of IgM antibodies of *L. donovani* in specimen. The result is positive

3) **IgG/IgM POSITIVE:** IgG and IgM Positive The control line (C), IgM (M) and IgG line (G) are visible on the test device. This is positive for both IgM and IgG antibodies to This indicates the presence of IgG antibodies of *L. donovani*



4) **NEGATIVE:** One distinct red line appears. The control line (c) is the only line visible on the test cassette. No IgM/IgG antibodies were detected. The result does not exclude This indicates the presence of IgG antibodies of *L. donovani* infection.

5) **INVALID:** Control line fails to appear. The test results are INVALID, if no control line (C) is visible, regardless of the presence or absence of line in the IgM(M)/IgG(G) region of the cassette. Repeat the test using a new cassette.



Quality Control

Internal procedural controls are included in the test individually. A colored line appearing in control line region (C) is the 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 of the Test

1. The test procedure, precautions and interpretation of results for this test must be followed when testing.
2. The Leishmania IgG/IgM Ab Rapid Test (Whole Blood/Serum/Plasma) is for in vitro diagnostic use only. This test should be used for detection of antibodies for *L. donovani* in whole blood, serum or plasma specimens. Neither the quantitative value nor the rate of increase in the concentration of *L. donovani* can be determined by this qualitative test.
3. A negative result can occur if the quantity of the anti- *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. Other clinically available tests are required. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

Performance characteristics

A total of 273 specimens were collected (including susceptible subjects) and tested by Leishmania IgG/IgM Ab Rapid Test and a commercial Leishmania IgG/IgM Ab Rapid Test as reference. Comparison for all subjects is showed in the following table.

	Clinical performance for IgM Test		
IgM Rapid Test	Positive	Negative	Total
Positive	26	0	26
Negative	2	223	225
Total	28	223	251

	Clinical performance for IgG Test		
IgG EIA	Positive	Negative	Total
Positive	22	0	22
Negative	2	223	225
Total	23	224	247

IgM Relative Sensitivity: 100%, IgG Relative Sensitivity: 100%, Relative Specificity: 99.11%, Overall Agreement: 98.9%

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.

GLOSSARY OF SYMBOL

	Consult Instruction for Use
REF	Catalog Number
	Store between
	Manufacturer
	Keep away from sunlight

Paramcare Life Sciences Private Limited, G/F-12/13, Evershine-2, Survey No. 307/3/1, Balitha N.H No 48, Vapi, Valsad, Gujarat, 396191.

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