OnSite® Leishmania Ab Rapid Test

REF R0122S CE

Instructions for Use

INTENDED USE

The *OnSite* Leishmania Ab Rapid Test is a lateral flow immunoassay for the qualitative detection of antibodies including IgG and IgM to the subspecies of *Leishmania donovani (L. donovani)*, the visceral leishmaniasis causative protozoans in human serum or plasma. It is intended to be used by professionals to aid in the diagnosis of the disease of visceral leishmaniasis.

Any use or interpretation of this preliminary test result must also rely on other clinical findings and the professional judgment of health care providers. Alternative test method(s) should be considered to confirm the test result obtained by this device.

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 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[6-7].

The *OnSite* Leishmania Ab Rapid Test is a recombinant protein based serological test, which detects antibodies including IgG and IgM to the *L. donovani*. The test provides a reliable result within 10-15 minutes without any instrumentation requirements.

TEST PRINCIPLE

The *OnSite* Leishmania Ab Rapid Test is a lateral flow chromatographic immunoassay. The test strip consists of: 1) a colored conjugate pad containing protein A conjugated with colloidal gold (Protein A conjugates) and a control antibody conjugated with colloidal gold, 2) a nitrocellulose membrane strip containing a test line (T line) and a control line (C line). The T line is pre-coated with a proprietary recombinant *L. donovani* antigen 39, and the C line is pre-coated with a control line antibody.

When an adequate volume of test specimen is dispensed into the sample pad of the strip, the specimen migrates by capillary action across the strip. Anti-*L. donovani* Ab if present in the specimen will bind to the Protein A conjugates. The immunocomplex is then captured on the membrane by the pre-coated antigen, forming a colored T line, indicating a *L. donovani* Ab positive test result.

Absence of the T line suggests a negative result. The test contains an internal control (C line) which should exhibit a colored line of the immunocomplex of control line antibodies regardless of any color development on the T line. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One strip device
   b. One desiccant
2. 5 µL capillary tubes
3. Sample diluent (REF SB-R0122, 5 mL/bottle)
4. Instructions for Use

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

1. Positive Control
2. Negative Control

MATERIALS REQUIRED BUT NOT PROVIDED

1. Clock or timer
2. A container for holding test specimen

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. Read these Instructions for Use completely before performing the test. Failure to follow the instructions could lead to 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 (15-30°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 hemolyzed blood specimen for testing.
7. Wear protective clothing and disposable gloves while handling the kit's reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. 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's 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 test results should be read 15 minutes after a specimen is applied to the sample well or sample pad of the device. Any results interpreted outside of the 15 minutes window should be considered invalid and must be repeated.
13. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test device unopened at 2-30°C. If stored at 2-8°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 to temperature above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Plasma/Serum

Step 1: Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.
Step 2: To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.
Step 3: To make serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately. The specimens can be stored at 2-8°C for 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 with result interpretation.

ASSAY PROCEDURE

Step 1: Bring the specimen and test components to room temperature, if refrigerated or frozen. Once thawed, mix the specimen well prior to performing the assay.

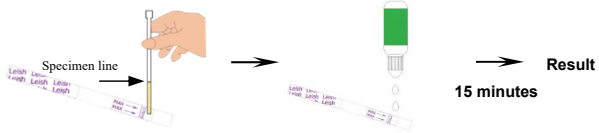
Step 2: When ready to test, open the pouch at the notch and remove the test strip and place it on a flat, dry surface.

Step 3: Fill the capillary tube with the specimen not to exceed the specimen line as shown in the following image. The volume of the specimen is around 5 µL.

Note: Practice a few times prior to testing if you are not familiar with the capillary tube. For better precision, transfer specimen by pipette capable of delivering 5 µL of volume.

Holding the capillary tube vertically, dispense all of the specimen into the sample pad making sure that there are no air bubbles.

Then immediately add 2 drops (about 70-100 µL) of sample diluent into the center of the sample pad with the bottle positioned vertically.



5 µL of specimen **2 drops of sample diluent**

Step 4: Set up the timer.

Step 5: Results should be read at 15 minutes. Positive results may be visible in as short as 1 minute. Negative results must be confirmed at the end of the 15 minutes only. ***Any results interpreted outside of the 15 minutes window should be considered invalid and must be repeated. Discard used devices after interpreting the result following local requirements governing the disposal of devices.***

QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding specimen and sample diluent. Otherwise, review the whole procedure and repeat test with a new device.

2. **External Control:** Good Laboratory Practice recommends using external controls, positive and negative, to assure the proper performance of the assay, particularly under the following circumstances:
   a. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of kits is used.
   d. The temperature during storage of the kit falls outside of 2-30°C.
   e. The temperature of the test area falls outside of 15-30°C.
   f. To verify a higher than expected frequency of positive or negative results.
   g. To investigate the cause of repeated invalid results.

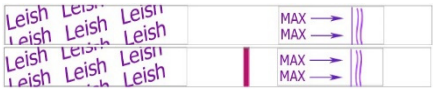
INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT**: If only the C line develops, the test indicates that no detectable anti- *L. donovani* Ab is present in the specimen. The result is negative or non-reactive.

2. **POSITIVE RESULT**: If both the C and the T lines develop, the test indicates for the presence of anti- *L. donovani* Ab in the specimen. The result is positive or reactive.

Specimens with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnosis decision is made.

3. **INVALID:** If no C line develops, the assay is invalid regardless of color development on the T line as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. **Clinical Performance**

A total of 200 specimens were collected from susceptible subjects and tested by the *OnSite* Leishmania Ab Rapid Test and by a reference Kalazar Ab rapid test. Comparison for all subjects is shown in the following table:

	***OnSite* Leishmania Ab Rapid Test**		
Reference	**Positive**	**Negative**	**Total**
Positive	31	0	31
Negative	0	169	169
Total	31	169	200

Relative Sensitivity: 100% (95% CI: 92.0% - 100%)
Relative Specificity: 100% (95% CI: 98.4% - 100%)
Overall Agreement: 100% (95% CI: 98.7% - 100%)

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 or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *OnSite* Leishmania Ab Rapid Test is limited to the qualitative detection of antibodies to *L. donovani* in human serum or plasma. The intensity of the test line does not have linear correlation with the antibody titer of the specimen
3. A negative or non-reactive 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 or non-reactive 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 in which a sample is collected.
5. Infection may progress rapidly. If the symptoms persist while the result from *OnSite* Leishmania Ab Rapid Test is negative or non-reactive, it is recommended to test with an alternative method.
6. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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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 CE Symbols

	Consult instructions for use	IVD	For *in vitro* diagnostic use only		Use by
REF	Catalog #	LOT	Lot Number	N	Tests per kit
2°C 30°C	Store between 2-30°C	EC REP	Authorized Representative		Do not reuse
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
13855 Stowe Drive
Poway, CA 92064, USA
Tel: 858-457-8698
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PI-R0122S Rev. F2.0
Date released: 2020-09-28
English version

For Export Only, Not For Re-sale in the USA

EC REP **MDSS GmbH**
Schiffgraben 41
30175 Hannover, Germany