OnSite Leishmania IgG/IgM Combo Rapid Test - (Serum / Plasma / Whole Blood)

CE

CTK BIOTECH SIMPLIFYING DIAGNOSTICS

REF

Catalog Number R0121C

IVD

In vitro Diagnostic

INTENDED USE

The *OnSite* Leishmania IgG/IgM Combo Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of IgG and IgM to the subspecies of *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 *OnSite* Leishmania IgG/IgM Combo Rapid Test must be confirmed with alternative testing method(s).

SUMMARY AND EXPLANATION OF THE TEST

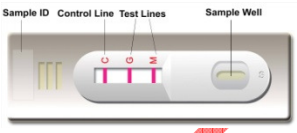
Visceral leishmaniasis, or Kala-azar, is a disseminated infection caused by several subspecies of *L. donovani*. The disease is estimated by the World Health Organization (WHO) to affect approximately 12 million people in 88 countries[1]. It is transmitted to humans by bites of the *Phlebotomus* sandflies 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* organisms from the blood, bone marrow, liver, lymph nodes or the spleen provides a definite means of diagnosis. Serological detection of anti-*L. donovani* IgM is found to be an excellent marker for acute visceral leishmaniasis. Tests used in the clinic include 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 *OnSite* Leishmania IgG/IgM Combo Rapid Test is a recombinant rK39 based serological test, which detects IgG and IgM antibodies to *L. donovani* simultaneously. The test provides a reliable result within 15 minutes without any instruments.

TEST PRINCIPLE

The *OnSite* Leishmania IgG/IgM Combo Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant rK39 antigen conjugated with colloidal gold (Leishmania conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing two test lines (M and G lines) and a control line (C line). The M line is pre-coated with monoclonal anti-human IgM for the detection of anti-*L. donovani* IgM, the G line is pre-coated with reagents for the detection of anti-*L. donovani* IgG, and the C line 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. *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 M line, indicating a *L. donovani* IgM positive test result.

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 G line, indicating a *L. donovani* IgG positive test result.

Absence of any test lines (M and G) suggests a negative result. The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of goat anti-rabbit IgG/rabbit IgG-gold conjugates regardless of color development on any of the test lines. If the C line does not develop, 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 cassette device
   b. One desiccant
2. Plastic droppers
3. Sample diluent (1 bottle, 5 mL)
4. One package insert (instruction 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. Lancing device for whole blood test
3. Pipette and tips capable of delivering 20 µL volumes with a precision better than 1.5%

WARNINGS AND PRECAUTIONS

For *in Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives 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°C - 30°C) before use.
5. Do not use components from any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolized blood specimens for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Users of this test should 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 reagents are being handled.
10. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
11. Handle the negative and positive controls in the same manner as patient specimens.
12. The test results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Reading the results after 15 minutes may give erroneous results.
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 devices 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.

SPECIMEN COLLECTION AND HANDLING

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

Plasma

Step 1: Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by veinpuncture.
Step 2: Separate the plasma by centrifugation.
Step 3: Carefully withdraw the plasma into new pre-labeled tube.

Serum

Step 1: Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by veinpuncture.
Step 2: Allow the blood to clot.
Step 3: Separate the serum by centrifugation.
Step 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 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 on result interpretation.

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.

ASSAY PROCEDURE

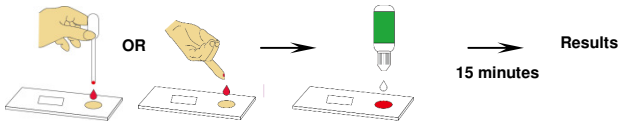
Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well, prior to assay, once thawed.

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

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

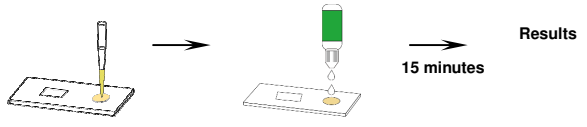
Step 4: **For whole blood test**
Apply 1 drop of whole blood (about 40-50 µL) into the sample well.

Then add 1 drop (about 35-50 µL) of Sample Diluent immediately.



For serum or plasma test
Dispense 20 µL of the specimen into the sample well.

Then add 2 drops (about 70-100 µL) of Sample Diluent immediately.



Step 5: Set up timer.

Step 6: Results can be read in 15 minutes. Positive results can be visible in as soon as 1 minute.

Do not read the results after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

QUALITY CONTROL

1. **Internal Control:** This test contains a built-in control feature, the C line. The C line develops after adding the specimen and the sample diluent. If the C line does not develop, review the whole procedure and repeat the 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. New operator uses the kit, prior to performing the testing of specimens.
   b. A new lot of test kits is used.
   c. A new shipment of kits is used.
   d. The temperature used during storage of the kits fall outside of 2°C - 30°C.
   e. The temperature of the test area falls outside of 15°C - 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

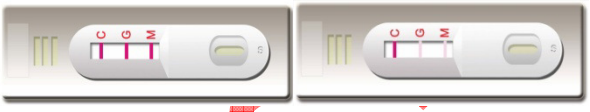
1. NEGATIVE RESULT: If only the C line is present, the absence of any burgundy color in both test lines (M and G) indicates that no anti-*L. donovani* antibody is detected in the specimen. The result is non-reactive.

2. POSITIVE RESULT:

2.1 In addition to the presence of the C line, if only the M line is developed, the test indicates the presence of IgM anti-*L. donovani* in the specimen. The result is IgM positive or reactive.

2.2 In addition to the presence of the C line, if only the G line is developed, the test indicates the presence of IgG anti-*L. donovani* in the specimen. The result is IgG positve or reactive.

2.3 In addition to the presence of the C line, if both the M and the G lines are developed, the test indicates the presence of both IgG and IgM anti-*L. Donovani* in the specimen. The result is also positive or reactive.



Samples with reactive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

3. INVALID: If no C line is developed, the assay is invalid regardless of any burgundy color in the test lines as indicated below. Repeat the assay with a new device.

PERFORMANCE CHARACTERISTICS

1. Clinical Performance For IgM Test

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

	OnSite Leishmania IgG/IgM Combo Rapid Test		
IgM EIA	**Positive**	**Negative**	**Total**
Positive	31	3	34
Negative	1	199	200
Total	32	202	234

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

2. Clinical Performance For IgG Test

A total of 214 samples from susceptible subjects were tested by the *OnSite* Leishmania IgG/IgM Combo Rapid Test and by a commercial *L. donovani* IgG EIA kit. Comparison for all subjects is shown in the following table.

	OnSite Leishmania IgG/IgM Combo Rapid Test		
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%

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Assay Result must be followed closely when testing the presence of antibodies to *L. donovani* in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *OnSite* Leishmania IgG/IgM Combo Rapid Test is limited to the qualitative detection of antibodies to *L. donovani* in human serum, plasma or whole blood. The intensity of the test line does not have a linear correlation with the antibody titer in the specimen.
3. A non-reactive result for an individual subject indicates absence of detectable anti-*L. donovani* antibodies. However, a non-reactive test result does not preclude the possibility of exposure tothe visceral leishmaniasis causative species of *L. donovani*.
4. A non-reactive 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. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
6. If the symptoms persist while the result from *OnSite* Leishmania IgG/IgM Combo Rapid Test is non-reactive, it is recommended to re-sample the patient a few days later or test with an alternative test method such as ELISA.
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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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
30°C 2°C	Store between 2-30°C	EC REP	Authorized Representative	2	Do not reuse
	Manufacturer		Date of manufacture		

CTK Biotech, Inc.
10110 Mesa Rim Road,
San Diego, CA 92121, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

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

PI-R0121C Rev. E
Effective date: 2013-06-11
English Version