Leishmania IgG/IgM Rapid Test- (Serum / Plasma)

DIAGNOCURE (INDIA)™ In vitro Diagnostic

INTENDED USE

The 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 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 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 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 Combo Rapid Test is a recombinant rK39 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.

TEST PRINCIPLE

The 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 colloid gold (Leishmania conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing two test bands (M and G bands) and a control band (C band). The M band is pre-coated with monoclonal anti-human IgM for the detection of anti-*L. donovani* IgM, G 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 M 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 G band, indicating a *L. donovani* IgG positive test result.

Absence of any test bands (M and G) 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 test bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Each kit contains 25 or 30 test devices, each sealed in a foil pouch with 3 items inside:
   a. One cassette device.
   b. One plastic dropper.
   c. One desiccant.
2. Sample diluent (3 bottle, 2.5 mL)
3. 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

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 the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolized blood specimen 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 biohazardous waste.
11. Handle the Negative and Positive Control in the same manner as patient specimens.
12. 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.
13. Do not perform the test in a room with strong air flow, ie. 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. The positive and negative controls should be kept at 2°C-8°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 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 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 device. Place the test device on a clean, flat surface.

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

For serum or plasma test

Dispense 1 drop (30-35 µL) of the specimen into the sample well,

Then add 1 drop (about 30 -35 µL) of Sample Diluent immediately

1 drop of serum/plasma 1 drop of sample diluent wait for 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.

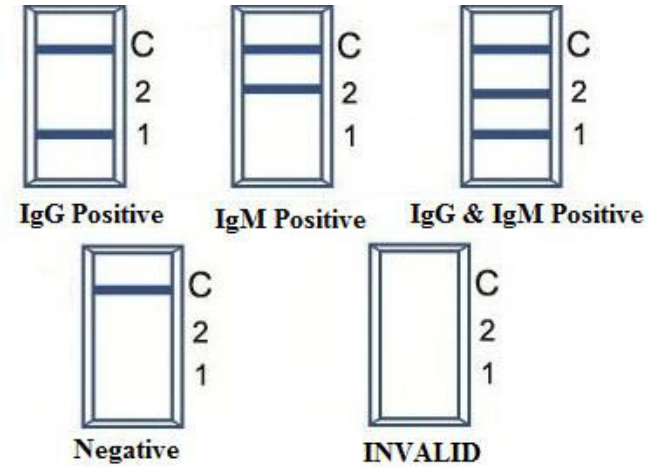
Don't read result after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

QUALITY CONTROL

Using individual Leishmania IgG/IgM Combo Rapid Test cassettes as described in the Assay Procedure above, run 1 Positive Control and 1 Negative Control under the following circumstances to monitor test performance:

1. A new operator uses the kit, prior to performing testing of specimens.
2. A new test kit is used.
3. A new shipment of kits is used.
4. The temperature used during storage of the kit falls outside of 2°C-30°C.
5. The temperature of the test area falls outside of 15°C-30°C.

INTERPRETATION OF ASSAY RESULT



PERFORMANCE CHARACTERISTICS

1. Clinical Performance For IgM Test

A total of 234 samples from susceptible subjects were tested by the 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.

	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 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.

	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 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 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 band does not have 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 to *Visceral leishmaniasis* causative species of the *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 titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. If the symptom persists, while the result from Leishmania IgG/IgM Combo Rapid Test is non-reactive result, it is recommended to re-sample the patient few days late or test with an alternative test methods such as ELISA.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical finding.

DIAGNOCURE (INDIA)™
2 F/F, E. C. Chambaghat, Solan, Himachal Pradesh -173213.
Cutomer Care No. +91-1792-230156
E-mail: diagnocure.india@gmail.com