INTENDED USE

The Leishmania Ab Rapid Test is a lateral flow immunoassay for the qualitative detection of antibodies including IgG and IgM to the subspecies of the Leishmania donovani (L. donovani), the Visceral leishmaniasis causative protozoan, in human serum or plasma. This test is intended to be used as a screening test and as an aid in the diagnosis of the disease of Visceral leishmaniasis.

SUMMARY AND EXPLANATION OF THE TEST

The 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 instrument application requirements. 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. 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. 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. Recently, utilization of L. donovani specific protein in the test has improved the sensitivity and specificity dramatically.

TEST PRINCIPLE

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

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 burgundy colored T band, indicating a L. donovani Ab positive test result.

Absence of the T band suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of anti-protein A – Protein A conjugates regardless the presence of any antibodies to L. donovani antigen. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Test Strip
2. Sample Diluent
3. Sample Droppers
4. package insert

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 hemolyzed 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. Reading result 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.

STORAGE INSTRUCTIONS

Store unused test devices unopened 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.

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 test strip and place in on a flat, dry surface.

Step 3: Fill in the mini plastic dropper with the specimen not to exceed the file line as showed 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 mini dropper. For better precision, transfer specimen by pipette capable to deliver 5µL volume.

Holding the dropper vertically, dispense 5 µL of the specimen into the sample pad making sure that there are no air bubbles. Then add 2 drops (about 70-100 µL) of Sample Diluent immediately.

Step 4: Set up the timer.

Step 5: Read the test result in 15 minutes. Positive result could be visible as short as 1 minute.

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

INTERPRETATION OF ASSAY RESULT

1. NEGATIVE RESULT: If only the C band is developed, the test indicates that no detectable anti-L. donovani Ab is present in the specimen. The result is negative.

2. POSITIVE RESULT: If both C and T bands are developed, the test indicates for the presence of anti-L. donovani Ab in the specimen. The result is positive.

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

3. INVALID: If no C band is developed, the assay is invalid regardless of color development on the T band as indicated below. Repeat the assay with a new device.
PERFORMANCE CHARACTERISTICS

Clinical Performance

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

<table>
<thead>
<tr>
<th></th>
<th>Leishmania Ab Rapid Test</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>169</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>169</td>
</tr>
</tbody>
</table>

Relative Sensitivity: 100%, Relative Specificity: 100%, Overall Agreement: 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 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 band does not have linear correlation with the antibody titer of 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. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES