INTENDED USE:
The Leishmania IgG/IgM Combo Rapid Test is a lateral flow immunassay for the simultaneous detection and differentiation of IgG and IgM to the subtypes of the Leishmania donovani (L. donovani), the Visna/ma��us visceralis causative protozoa, 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 Visna/ma��us visceralis. Any reactive specimen with the Leishmania IgG/IgM Combo Rapid Test must be confirmed with alternative testing methods.

SUMMARY AND EXPLANATION:
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 82 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 found in poor countries, in Southern Europe it has become the leading opportunistic infection in AIDS patients.2 Identification of L. donovani organism from the blood, bone marrow, liver, lymph nodes or the spleen provides a definite means of diagnosis. Serological detection of anti-L. donovani IgG is found to be an excellent marker for the acute Visceral leishmaniasis. Tests used in clinics include ELISA, fluorescent antibody or direct agglutination tests.2, 4 Recently, utilization of L. donovani specific protein in the test has improved the specificity and sensitivity dramatically.3, 4

The Leishmania IgG/IgM Combo Rapid Test is a recombinant K39 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.

PRINCIPLE OF THE TEST:
The Leishmania IgG/IgM Combo Rapid Test is a lateral flow chromatographic immunassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant K39 antigen conjugated with colored 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 by the pre-coated reagents on the membrane, forming a burgundy colored M band, indicating a L. donovani IgM positive test result.

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Absence of any test bands (M band 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/anti 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:
1. Each kit contains 20 test devices, each sealed in a foil pouch with three items inside:
   a. One cassette device.
   b. One pipette dropper.
   c. One desiccant.
   2. Sample Driant (1 bottle, 3 mL).
   3. One package-insert (instruction for use).
   4. Positive Control (available on request).
   5. Negative Control (available on request).
   6. Clock or Timer (not provided).
   7. Lancing device for whole blood test (not provided).
   8. Pipette and tip caps capable of delivering 20 µl volumes with a precision better than 1.5% (not provided).

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 haemolysed blood specimen for testing.
7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens.
8. Wash hands thoroughly after performing the test.
9. Users of this test should follow the Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
10. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
11. Dispose of all specimens and materials used to perform the test as biohazardous waste.
12. Handle the Negative and Positive Control in the same manner as patient specimens.
13. 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 after 15 minutes may give erroneous results.
14. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.

REAGENT PREPARATION AND STABILITY:
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.

TYPE OF SPECIMEN:
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 venepuncture.
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 venepuncture.
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.

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 any finger tip puncture or venepuncture. Do not use any haemolysed blood for testing.
Whole blood specimens should be stored in refrigerator (2°C-8°C) if not tested immediately. The specimens must be tested within 24 hours of collection.

TEST 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.
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.

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-8°C.
5. The temperature of the test area falls outside of 15°C-30°C.
EXPECTED RESULTS:

1. NEGATIVE RESULT: If only the C band is present, the absence of any burgundy color in the both test bands (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 C band, if only M band is developed, the test indicates for the presence of IgM anti-L. donovani in the specimen. The result is reactive.

2.2 In addition to the presence of C band, if only G band is developed, the test indicates for the presence of IgG anti-L. donovani in the specimen. The result is also reactive.

2.3 In addition to the presence of C band, both M and G bands are developed, the test indicates for the presence of both IgG and IgM anti-L. donovani in the specimen. The result is also reactive.

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

PERFORMANCE CHARACTERISTICS:

1. Clinical Performance For IgM 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 IgM EIA. Comparison for all the subjects is shown in the following table.

<table>
<thead>
<tr>
<th>IgM EIA</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>31</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>199</td>
<td>200</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>202</td>
<td>234</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>IgG EIA</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>14</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>198</td>
<td>200</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>199</td>
<td>215</td>
</tr>
</tbody>
</table>

Relative Sensitivity: 92.9%, Relative Specificity: 99.0%, Overall Agreement: 98.6%

BIBLIOGRAPHY:


SYMBOLS:
The following symbols are used in the labelling of Audit Diagnostics systems:

- For in vitro diagnostic use only
- Use by
- Store between 2-30°C
- Authorized Representative
- Date of manufacture

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