OnSite™ Leptospira IgG/IgM Combo Rapid Test

INTENDED USE

The OnSite Leptospira IgG/IgM Combo Rapid Test is a lateral flow immunocassette for the simultaneous detection and differentiation of IgG and IgM antibodies to Leptospira interrogans (L. interrogans) in human serum, plasma or whole blood. It is intended to be used as a screening test by professionals, and provides a preliminary test result to aid in the diagnosis of infection with L. interrogans. 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 methods should be considered to confirm the test result obtained by this device.

SUMMARY AND EXPLANATION OF THE TEST

Leptospirosis occurs worldwide and is a common mild to severe health problem for humans and animals, particularly in areas with hot and humid climates. The natural reservoirs for leptospirosis are rodents as well as a large variety of domesticated mammals. Human infection is caused by L. interrogans, the pathogenic member of the genus Leptospira. The infection is spread via urine from the host animal.

After infection, leptospires are present in the blood until they are cleared approximately 4 to 7 days after the onset of the disease following the production of anti-L. interrogans antibodies. Antibodies to leptospires are detectable by the 6th to 10th day of disease and levels peak within 3 to 4 weeks and then gradually decline. Antibodies may be detectable for years post infection. IgM anti-leptospira are detectable during the first week of illness allowing early therapeutic intervention at a time point where it is most effective.

Sero logical detection of anti-L. interrogans antibodies (IgM and IgG) is a common diagnostic method. Tests available include: 1) The microscopic agglutination test (MAT); 2) ELISA; and 3) and Indirect fluorescent antibody tests (IFT). Isolation of leptospires by culture of blood, urine and cerebrospinal fluid samples is an effective means of confirming the diagnosis. However culture methods can only be performed within 15-20 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE

The OnSite Leptospira IgG/IgM Combo Rapid Test is a lateral flow chromatographic immunocassette. The test cassette consists of: 1) a burgundy colored conjugate pad containing L. interrogans antigens conjugated with colloidal gold (Leptospira conjugates) and a control antibody conjugated with colloidal gold; 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 monochlonal anti-human IgM for the detection of IgM anti-L. interrogans, G line is pre-coated with monochlonal anti-human IgG for the detection of IgG anti-L. interrogans, and the C line is pre-coated with a control line antibody.

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. IgM anti-L. interrogans, if present in the specimen, will bind to the Leptospira conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgG antibody forming a burgundy colored M line, indicating an IgM anti-L. interrogans positive test result. IgG anti-L. interrogans, if present in the specimen, will bind to the Leptospira conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-human IgM forming a burgundy colored G line, indicating an IgG anti-L. interrogans positive test result.

Absence of any test lines (M and G) suggests a negative test result. The test contains an internal control (C line) which should exhibit a burgundy colored line of the immunocomplex of the control antibodies 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.

TEST PROCEDURE

1. Individualized sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
   c. 5 µL capillary tubes
   d. Sample diluent (REF SB-R0101, 5 mL/Tube)
   e. One package insert (instruction for use)

2. MATERIALS MAY BE REQUIRED BUT NOT PROVIDED

   a. One clock or timer
   b. Lancing device for whole blood test

3. MATERIALS REQUIRED BUT NOT PROVIDED

   a. 5 µL serum/plasma to S well
   b. 2 drops of sample diluent to B well
   c. 5 µL whole blood to S well
   d. 2 drops of sample diluent to B well

4. ASSAY PROCEDURE

   Step 1: Bring the specimen and the 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 device. Place the device on a clean, flat surface.
   Step 3: Be sure to label the device with the specimen ID number.
   Step 4: Fill the 5 µL capillary tube with the specimen not to exceed the specimen line as shown in the following image. The volume of the specimen is approximately 5 µL. For maximum precision, transfer the specimen using a pipette capable of delivering a volume of 5 µL.
   Step 5: Hold the capillary tube vertically, dispense the entire specimen into the center of the sample well (S well) making sure that there are no air bubbles.
   Step 6: Immediately add 2 drops (approximately 60-80 µL) of sample diluent into the buffer well (B well) with the bottle positioned vertically.

   Step 7: Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
   Step 8: Use this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
   Step 9: Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
   Step 10: Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
   Step 11: Handle the negative and positive controls in the same manner as patient specimens.
   Step 12: The test results should be read 15-20 minutes after a specimen is applied to the sample well of the device. Any results interpreted outside of the 15-20 minute window should be considered invalid and must be repeated.
   Step 13: Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.

5. SPECIMEN COLLECTION AND HANDLING

   Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.
   a. Plasma
      1. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by venipuncture.
      2. Separate the plasma by centrifugation.
      3. Carefully withdraw the plasma into a new pre-labeled tube.
   b. Serum
      1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by venipuncture.
      2. Allow the blood to clot.
      3. Separate the serum by centrifugation.
      4. Carefully withdraw the serum into a new pre-labeled tube.
   c. Whole Blood
      1. Drops of whole blood can be obtained by different methods, such as finger tip puncture or venipuncture.
      2. Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®). Do not use hemolyzed blood for testing.

6. ASSAY PROCEDURE

   a. Step 1: Bring the specimen and the test components to room temperature if refrigerated or frozen. Once thawed, mix the specimen well prior to performing the assay.
   b. Step 2: When ready to test, open the pouch at the notch and remove the device. Place the device on a clean, flat surface.
   c. Step 3: Be sure to label the device with the specimen ID number.
   d. Step 4: Fill the 5 µL capillary tube with the specimen not to exceed the specimen line as shown in the following image. The volume of the specimen is approximately 5 µL. For maximum precision, transfer the specimen using a pipette capable of delivering a volume of 5 µL.
   e. Step 5: Hold the capillary tube vertically, dispense the entire specimen into the center of the sample well (S well) making sure that there are no air bubbles.
   f. Step 6: Immediately add 2 drops (approximately 60-80 µL) of sample diluent into the buffer well (B well) with the bottle positioned vertically.

7. WARNING AND PRECAUTIONS

   a. 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 temperatures above 30°C.
   b. Do not perform the test in a room with strong air flow, i.e. an electric fan or strong air-conditioning.
   c. Do not use hemolyzed blood specimens for testing.

8. PREPARATION AND STORAGE INSTRUCTIONS

   a. 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 temperatures above 30°C.
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. A new operator uses the kit, prior to performing testing of specimens.
   b. A new lot of test kit 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 is present, the absence of any burgundy color in both test lines (M and G) indicates that no detectable anti-L. interrogans antibody is present in the specimen. The result is negative or non-reactive.

2. POSITIVE RESULT:
   2.1 In addition to the presence of the C line, if only the M line develops, the test indicates the presence of IgG anti-L. interrogans. The result is IgG anti-L. interrogans positive or reactive.

   2.2 In addition to the presence of the C line, if only the G line develops, the test indicates the presence of IgM anti-L. interrogans. The result is IgM anti-L. interrogans positive or reactive.

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

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

3. INVALID: If no C line develops, 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. Accuracy of IgG Detection
   Specimens collected from suspicious patients and normal individuals were studied. The OnSite Leptospira IgG/IgM Combo Rapid Test shows 100% specificity and 100% sensitivity in comparison with a reference rapid test from the market.

2. Cross reactivity
   Specimens from other infectious diseases were tested for cross-reactivity with the OnSite Leptospira IgG/IgM Combo Rapid Test according to the standard procedure. The results showed that the following specimens (n=3-10) did not cross-react with the OnSite Leptospira IgG/IgM Combo Rapid Test:
   - HAV
   - HBV
   - HCV
   - HEV
   - H. pylori
   - NCG
   - HIV
   - Dengue
   - TB
   - P. tuliporum
   - Typhoid
   - ANA
   - HAMA
   - RF (up to 8,400 IU/mL)

3. Interference
   Common substances (such as pain and fever medication, blood components) may affect the performance of the OnSite Leptospira IgG/IgM Combo Rapid Test. This was studied by spiking these substances into negative and positive standard controls. The results are presented in the following table and demonstrate, at the concentrations tested, the substances studied do not affect the performance of the OnSite Leptospira IgG/IgM Combo Rapid Test.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>60 g/L</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Creatinine</td>
<td>442 µmol/L</td>
</tr>
<tr>
<td>EDTA</td>
<td>3.4 µmol/L</td>
</tr>
<tr>
<td>Glucose</td>
<td>55 mmol/L</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>2 g/L</td>
</tr>
<tr>
<td>Heparin</td>
<td>3,000 U/L</td>
</tr>
<tr>
<td>Human IgG</td>
<td>1000 mg/dL</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>4.34 mmol/L</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>3.8%</td>
</tr>
</tbody>
</table>

LIMITATIONS OF TEST:

1. The Assay Procedure and the Interpretation Assay Result sections must be followed closely when testing for the presence of antibodies to pathogenic L. interrogans in human serum, plasma or whole blood. An initial test line of the assay does not have a linear correlation with antibody titer in the specimen.

2. A negative result for an individual subject indicates absence of detectable L. interrogans antibodies. However, a negative test result does not preclude the possibility of exposure to L. interrogans. An invalid test result can occur if the quantity of L. interrogans 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.

3. Specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.

4. Depending on the circulating Leptospira aerovirginensis regionally present at the time of collection, specificity of this product may vary.

5. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES:


Date of manufacture: 2016-06-17