**OnSite Leptospira IgG/IgM Combo Rapid Test- Cassette (Serum / Plasma / Whole Blood)**

**INTENDED USE**

The OnSite Leptospira IgG/IgM Combo Rapid Test is a lateral flow immunomassay for the simultaneous detection and differentiation of IgM and IgG antibody to Leptospira interrogans (L. interrogans) 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 infection with L. interrogans. Any reactive specimen with the OnSite Leptospira IgG/IgM Combo Rapid Test must be confirmed with alternative testing method(s).

**SUMMARY AND EXPLANATION OF THE TEST**

Leptospiriosis occurs worldwide and is a common mild to severe health problem for humans and animals, particularly in areas with a hot and humid climate. 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 of Leptospira². The infection is spread via urine from the host animal.

After infection, leptospires are present in the blood until they are cleared after 4 to 7 days following the production of anti-L. interrogans antibodies. Initially of the IgM class. Culture of the blood, urine and cerebrospinal fluid is an effective means of confirming the diagnosis during the 1st to 2nd weeks after exposure. Serological detection of anti-L. interrogans antibodies is also a common diagnostic method. Tests are available under this category: 1) The microscopic agglutination test (MAT)²; 2) ELISA;² 3) Indirect fluorescent antibody tests (IFA)². However, all above mentioned methods require a sophisticated facility and well-trained technicians.

The OnSite Leptospira IgG/IgM is a simple serological test that utilizes antigens from L. interrogans and detects IgG and IgM antibodies to these microorganisms simultaneously. The test can be performed by untrained or minimally skilled personnel, without cumbersome laboratory equipment and the result is available within 15 minutes.

**TEST PRINCIPLE**

The OnSite Leptospira IgG/IgM Combo Rapid Test is a lateral flow chromatographic immunomassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant L. interrogans antigens conjugated with colloidal gold (Leptospira conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing two test bands (T1 and T2 bands) and a control band (C band). The T1 band is pre-coated with monoclonal anti-human IgM for the detection of anti-L. interrogans IgM, T2 band is pre-coated with reagents for the detection of anti-L. interrogans 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. 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 IgM antibody, forming a burgundy colored T1 band, indicating a L. interrogans IgM positive test result.

IgG anti-L. interrogans if present in the specimen will bind to the Leptospira conjugates. The immunocomplex is then captured by the pre-coated reagents on the membrane, forming a burgundy colored T2 band, indicating a L. interrogans IgG positive test result.

Absence of any T bands (T1 and T2) 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 T bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

**REAGENTS AND MATERIALS PROVIDED**

1. Each kit contains 30 test devices, each sealed in a foil pouch with three items inside:
   a. One cassette device.
   b. One plastic dropper.
   c. One desiccant.
2. Sample diluent (1 bottle, 5 mL)
3. One package insert (instruction for use).

**MATERIALS REQUIRED AND AVAILABLE PER REQUEST**

1. Positive Control (1 vial, red cap, 1 mL, Cat # R0101-P)
2. Negative Control (1 vial, green cap, 1 mL, Cat # R0101-N)

**MATERIALS REQUIRED BUT NOT PROVIDE**

1. Clock or Timer

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**WARNING 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.

**REAGENT PREPARATION AND STORAGE INSTRUCTIONS**

All reagents are ready to use as supplied. Store unused test device 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.

**SPECMEN 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. Allow the blood to clot.
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.

**Step 4:**

For whole blood test

1. Apply 1 drop of whole blood (about 40-50µL) into the sample well.
2. Lancing device for whole blood test

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

**For serum or plasma test**

Fill the pipette dropper with the specimen.

Holding the dropper vertically, dispense 1 drop (about 30-45µL) of specimen into the sample well making sure that there are no air bubbles.
Then add 1 drop (about 35-50 µL) of Sample Diluent immediately.

1 drop of specimen 1 drop of sample diluent 15 minutes

1. **Slap 5:** Set up timer.
2. **Slap 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 OnSite Leptospira IgG/IgM Combo Rapid Test cassettes as described in the Assay Procedure above, run 1 Positive Control and 1 Negative Control (provided upon request) 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.

Expected results are as follows:

**Negative Control**

Only the C band shows color development, the two T bands (T1 and T2) show no color development.

**Positive Control**

The C band and two T bands (T1 and T2) show color development.

The appearance of any burgundy color in the T bands, regardless of intensity, must be considered as presence of the band.

### INTERPRETATION OF ASSAY RESULT

1. **NEGATIVE RESULT:** If only the C band is present, the absence of any burgundy color in the both T bands (T1 and T2) indicates that no anti-L. interrogans antibody is detected. The result is negative.

2. **POSITIVE RESULT:**
   2.1 In addition to the presence of C band, if only T1 band is developed, the test indicates for the presence of IgM anti-L. interrogans. The result is positive.
   
   2.2 In addition to the presence of C band, if only T2 band is developed, the test indicates for the presence of IgG anti-L. interrogans. The result is positive.
   
   2.3 In addition to the presence of C band, both T1 and T2 bands are developed, the test indicates for the presence of both IgG and IgM anti-L. interrogans. The result is also positive.

Samples with positive results should be confirmed with alternative testing methods (or clinical findings before a positive determination is made).

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

### PERFORMANCE CHARACTERISTICS

#### 1. Clinical Performance For IgM Test

A total of 210 samples from susceptible subjects were tested by the OnSite Leptospira IgG/IgM Combo Rapid Test and by a commercial Leptospira IgM EIA kit. Comparison for all results is showed in the following table.

<table>
<thead>
<tr>
<th>OnSite Leptospira IgG/IgM Combo Rapid Test</th>
<th>IgM EIA Positive</th>
<th>IgM EIA Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>9</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>198</td>
<td>200</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>199</td>
<td>210</td>
</tr>
</tbody>
</table>

Relative Sensitivity: 90.0%, Relative Specificity: 99.0%, Overall Agreement: 98.6%

#### 2. Clinical Performance For IgG Test

A total of 206 samples from susceptible subjects were tested by the OnSite Leptospira IgG/IgM Combo Rapid Test and by a commercial Leptospira IgG EIA kit. Comparison for all subjects is showed in the following table.

<table>
<thead>
<tr>
<th>OnSite Leptospira IgG/IgM Combo Rapid Test</th>
<th>IgG EIA Positive</th>
<th>IgG EIA Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>198</td>
<td>200</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>198</td>
<td>206</td>
</tr>
</tbody>
</table>

Relative Sensitivity: 100%, Relative Specificity: 99.0%, Overall Agreement: 99.0%

### LIMITATIONS OF TEST

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of antibodies to pathogenic L. interrogans in serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.

2. The OnSite Leptospira IgG/IgM Combo Rapid Test is limited to the qualitative detection of antibodies to L. interrogans in human serum, plasma or whole blood. The intensity of the test band may not show linear correlation with antibody titer in the specimen.

3. 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.

4. A negative 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.

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