LEPTOSPIRA TEST PROCEDURE:

ADRT0101 1 X 20 Tests

IGG/IGM COMBO

1 x 3 ml Diluent

1 drop of whole blood              1 drop of sample diluent

3. One package insert (Instruction for use).

5. Negative Control (not provided).

7. Lancing device for whole blood test (not provided).

INTENDED USE:

The Leptospira IgG/IgM Combo Rapid Test is a lateral flow immunoassay for the simultaneous detection and differentiation of IgG and IgM 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 Leptospira IgG/IgM Combo Rapid Test must be confirmed with alternative testing methods.

SUMMARY AND EXPLANATION:

Leptospirosis 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 Leptospira 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 *Leptospira*. 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 week 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) ELISA; 3) Indirect fluorescent antibody test (IFAT). However, all above mentioned methods require a sophisticated facility and well-trained technicians.

The Leptospira IgG/IgM rapid test 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.

PRINCIPLE OF THE TEST:

The Leptospira IgG/IgM Combo Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy coloured 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 (M and G) and a control band (C). The M band is pre-coated with monoclonal anti-human IgG for the detection of anti-*L. interrogans* IgG, G band is pre-coated with reagents for the detection of anti-*L. interrogans* IgM, 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. 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 IgG antibody, forming a burgundy coloured M band, indicating a *L. interrogans* 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 coloured band of the immunocomplex of goat anti rabbit IgG/rabbit IgG-gold conjugate regardless of the colour development on any of the test bands. Otherwise, the test result is invalid and the specimen must be rejected with another device.

### REAGENTS AND MATERIALS REQUIRED:

1. Each kit contains 20 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, 3 mL).
3. One package insert (Instruction for use).
4. Positive Control (not provided).
5. Negative Control (not provided).
6. Clock or Timer (not provided).
7. Lancing device for whole blood test (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 haemolized 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. 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 results 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.

### REAGENT PREPARATION AND STABILITY:

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 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 vein puncture.
2. Carefully withdraw the plasma into new pre-labelled tube.

**Serum**

1. Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer®) by vein puncture.
2. Allow the blood to clot.
3. Carefully withdraw the serum into a new pre-labelled tube.

**Test specimen**

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.

**Blood**

Drops of whole blood can be obtained by either finger tip puncture or vein puncture. Do not use any haemolized blood for testing.

Whole blood specimens should be stored in refrigeration (2°C-6°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.

**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. Do not read result after 15 minutes. To avoid confusion, discard the test device after interpreting the result.

### QUALITY CONTROL:

Using individual Leptospira IgG/IgM Combo Rapid Test cassettes as described in the assay procedure above, run 1 Positive Control and 1 Negative Control (not provided) 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:

1. NEGATIVE RESULT: If only the C band is present, the absence of any burgundy colour in both test bands (M and G) 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 M 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 G 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 M and G 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 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 any burgundy colour 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 210 samples from susceptible subjects were tested by the Leptospira IgG/IgM Combo Rapid Test and by a commercial Leptospira IgM EIA kit. Comparison for all 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>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 Leptospira IgG/IgM Combo Rapid Test and by a commercial Leptospira 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>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%

BIBLIOGRAPHY:


SYMBOLS:

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

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