INTENDED USE

The Leptospira IgG/IgM Combo Rapid Test is a lateral flow immunassay for the simultaneous detection and identification of Leptospira interrogans (L. interrogans) IgG and IgM antibodies 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 method(s) 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 of 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. Anti-L. interrogans IgM are detectable during the first week of illness allowing early therapeutic intervention at a time point where it is most effective. IgG class antibodies appear at a later time point of infection and may persist for several years.

Serological 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) Indirect fluorescent antibody tests (IFATs). 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 applied at the early stages of infection in the 1st and 2nd weeks after exposure and leptospirosis ends by the first week of illness. All above mentioned methods require a sophisticated facility and well-trained technicians.

The Leptospira IgG/IgM Combo Rapid Test is a simple serological test that utilizes antigens from L. interrogans to detect and differentiate IgG and IgM antibodies to L. interrogans. The test can be performed within 15 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE

The Leptospira IgG/IgM Combo Rapid Test is a lateral flow chromatographic immunassay. 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 C line is pre-coated with monoclonal anti-human IgM for the detection of anti-L. interrogans IgM, G line is pre-coated with monoclonal anti-human IgG for the detection of anti-L. interrogans IgG, 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. Anti-L. interrogans IgG, 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 forming a burgundy colored G line, indicating an anti-L. interrogans IgG positive test result.

Absence of any test lines (M and G) suggests a negative result. The test contains an internal control (C line) which does not 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 restested with another device.

TEST PROCEDURE

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
   c. 5 µL capillary tubes
   d. Sample diluent (REF SB-R0101, 5 mL/bottle)
   e. One package insert (instruction for use)
   f. One 2-hole capillary tube

2. Place the device on a flat, clean surface.

3. Set up timer.

4. Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.

5. Once thawed, mix the specimen well prior to performing the assay.

6. Do not use hemolyzed blood specimens 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 bio-hazardous waste.

11. Handle the negative and positive controls in the same manner as patient specimens.

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.

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 STORAGE INSTRUCTIONS

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 it to temperatures above 30°C.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety procedures.

Plasma/Serum

Step 1: Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.

Step 2: To make plasma specimen, centrifuge collected specimens and carefully withdraw the plasma into a new pre-labeled tube.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately.

Step 3: To make serum specimen, allow blood to clot, then centrifuge collected specimens and carefully withdraw the serum into a new pre-labeled tube.

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 with result interpretation.

Whole Blood

Step 1: Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture. Collect blood specimen into a collection tube containing EDTA, citrate or heparin. Do not use hemolyzed blood for testing.

Whole blood specimens should be stored in refrigeration (2-8°C), if not tested immediately. The specimens must be tested within 24 hours of collection.

ASSAY PROCEDURE

1. Collect blood specimen into collection tube containing EDTA, citrate or heparin for plasma or collection tube containing no anticoagulants for serum by venipuncture.

2. When ready to test, open the pouch at the notch and remove the device. Place the device on a clean, flat surface.

3. Be sure to label the device with the specimen’s ID number.

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.

5. Holding the capillary tube vertically, dispense the entire specimen into the center of the specimen well (S well) making sure that there are no air bubbles. Immediately add 2 drops (approximately 60-80 µL) of sample diluent into the buffer well (B well) with the bottle positioned vertically.

6. Place the device on a flat, clean surface.

7. Set up timer.

8. Results can be read at 15 minutes. Positive results can be visible in as soon as 1 minute. Negative results must be confirmed at the end of the 20 minutes only. Any results interpreted outside of the 15-20 minute window should be considered invalid and must be repeated. Discard used devices after interpreting the result following local requirements governing the disposal of devices.
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 antibodies are present in the specimen. The result is negative or non-reactive.

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

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

   2.3 In addition to the presence of the C line, both the M and the G lines develop, the test indicates the presence of both anti-L. interrogans IgG and IgM. The result is both anti-L. interrogans IgG and IgM 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 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 Leptospira IgG/IgM Combo Rapid Test according to the standard procedure. The results showed that the following specimens (n=10) did not cross-react with the Leptospira IgG/IgM Combo Rapid Test.
   - HAV
   - HBV
   - HCV
   - H. pylori
   - HCG
   - HSV
   - Danque
   - TB
   - T. pallidum
   - Typhoid
   - ANA
   - HAMA
   - RF (up to 8,400 IU/mL)

   List of potentially interfering substances and concentrations tested:
   - Albumin: 60 g/L
   - Hemoglobin: 2 g/L
   - Bilirubin: 20 mg/dL
   - Heparin: 3,000 U/L
   - Creatinine: 442 μmol/L
   - Human IgG: 1000 mg/dL
   - EDTA: 9 mL
   - Sodium Citrate: 3.8%