Leptospira IgG/IgM Rapid Test - (Serum / Plasma / Whole Blood)

BIOGENIX INC. PVT. LTD.

IVD **In Vitro Diagnostic**

INTENDED USE

The Leptospira IgG/IgM Cassette Rapid Test is a lateral flow immunoassay 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 and as an aid in the diagnosis of infection with L. interrogans. Any reactive specimen with the Leptospira IgG/IgM Cassette Rapid Test must be confirmed with alternative testing method(s).

SUMMARY AND EXPLANATION OF THE TEST

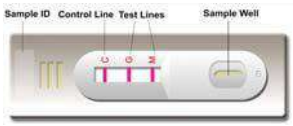
Leptospirosis occurs worldwide and is a common mild to severe health problem for humans and animals, particularly in areas with hot and humid climates. The natural reservoirs for leptospirosis are rodents as well as a large variety of domesticated mammals. Human infection is caused by L. interrogans, the pathogenic member of the genus of Leptospira1, 2. 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, initially of the IgM class. Culture of the blood, urine and cerebrospinal fluid is an effective means of confirming the diagnosis during the 1st and 2nd weeks after exposure. Serological detection of anti-L. interrogans antibodies is also a common diagnostic method. Tests available under this category include: 1) The microscopic agglutination test (MAT) 3; 2) ELISA4-5; 3) Indirect fluorescent antibody tests (IFATs) 6. However, all above mentioned methods require a sophisticated facility and well-trained technicians.

The Leptospira IgG/IgM Cassette Rapid Testt 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 Leptospira IgG/IgM Cassette Rapid Test is a lateral flow chromatographic immunoassay. 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 lines (M and G lines) and a control line (C line). The M line is pre-coated with monoclonal anti-human IgM for the detection of anti-L. interrogans IgM, G line is pre-coated with reagents for the detection of anti-L. interrogans IgG, and the C line 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 M line, 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 on the membrane by the pre-coated reagents forming a burgundy colored G line, indicating a 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 should exhibit a burgundy colored line of the immunocomplex of goat anti-rabbit IgG/rabbit IgG-gold conjugates 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.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
a. One cassette device
b. One desiccant
2. Capillary tubes
3. Sample diluent (1 bottle, 5 ml)
4. One package insert (instruction for use)

MATERIALS MAY BE REQUIRED AND NOT PROVIDED

1. Clock or Timer
2. Lancing device for whole blood test

MATERIALS REQUIRED BUT NOT PROVIDED

1.Clock or Timer
2.Lancing device for whole blood test

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
6. components in this kit.
7. Do not use hemolized blood specimens for testing.
8. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
9. Users of this test should follow the US CDC 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 bio-hazardous waste.
12. Handle the negative and positive controls in the same manner as patient specimens.
13. The test results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Reading the results 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 STORAGE INSTRUCTIONS

All reagents are ready to use as supplied. Store unused test device unopened at 2°C - 30°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 to over 30°C.

SPECIMEN COLLECTION AND HANDLING

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

Plasma

Step 1: Collect blood specimen into a lavender, blue or green top collection tube (containing EDTA, citrate or heparin, respectively, in Vacutainer®) by veinpuncture.
Step 2: Separate the plasma by centrifugation.
Step 3: Carefully withdraw the plasma into a new pre-labeled tube.

Serum

Step 1: Collect blood specimen into a red top collection tube (containing no anticoagulants in Vacutainer) by veinpuncture.
Step 2: Allow the blood to clot.
Step 3: Separate the serum by centrifugation.
Step 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 for 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 lipermia, gross hemolysis or turbidity in order to avoid interference on result interpretation.

Blood

Drops of whole blood can be obtained by either fingertip puncture or veinpuncture. Do not use 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

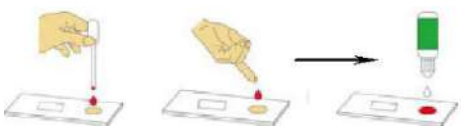
Step 1: Bring the specimen and the test components to room temperature if refrigerated or frozen. Mix the specimen well, prior to assay, once thawed.
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the test device on a clean, flat surface.
Step 3: Be sure to label the device with the specimen's ID number.
Step 4: Fill the plastic dropper with the specimen.

Holding the dropper vertically, dispense 1 drop (about 30 -45 µL) of serum/plasma or 1 drop of whole blood (about 40-50 µL) into the sample well making sure that there are no air bubbles.

Immediately add 1 drop (about 35-50 µL) of Sample Diluent to the sample well.

1 drop of specimen **1 drop of sample diluent**

Result 15 minutes



1 drop of specimen **1 drop of sample diluent**

Step 5: Set up timer.
Step 6: Results can be read in 15 minutes. Positive results can be visible in as soon as 1 minute.

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

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. New operator uses the kit, prior to performing the testing of specimens.
b. A new lot of test kits are used.
c. A new shipment of test kits is used.
d. The temperature used during storage of the kits fall outside of 2°C - 30°C.
e. The temperature of the test area falls outside of 15°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 anti-L. interrogans antibody is detected. The result is negative.

2. **POSITIVE RESULT:**

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

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

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 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 line is developed, 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. 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 of the results for all subjects is shown in the following table.

Leptospira IgG/IgM Cassette Rapid Test			
IgM EIA	**Positive**	**Negative**	**Total**
Positive	9	1	10
Negative	2	198	200
Total	11	199	210

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 qLeptospira IgG/IgM Combo Rapid Test and by a commercial Leptospira IgG EIA kit. Comparison of the results for all subjects is shown in the following table.

Leptospira IgG/IgM Cassette Rapid Test			
IgG EIA	**Positive**	**Negative**	**Total**
Positive	6	0	6
Negative	2	198	200
Total	8	198	206

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

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 serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Leptospira IgG/IgM Cassette 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 line does not have a 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 titers 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

1. Stallman GND. The International Committee on Systematic Bacteriology: Subcommittee on the Taxonomy of Leptospira. Int J Syst Bacteriol 1987; 37:472.
2. Levett PN. Leptospirosis. Clin Microbiol Rev 2001;14:296-326
3. Faine S, ed. Guidelines for the control of leptospirasis. Geneva: World Health Organization, 1982.
4. Cumberland PC, Everard COR, Levett PN. Assessment of the efficacy of the IgM enzyme-linked immunosorbent assay (ELISA) and microscopic agglutination test (MAT) in the diagnosis of acute leptospirosis. Am J Trop Med Hyg. 1999; 61: 731–734.
5. Adler B, Murphy AM, Locarnini SA, Faine S. Detection of specific anti-leptospiral immunoglobulins M and G in human serum by solid-phase enzyme-linked immunosorbent assay. J Clin Microbiol. 1980; 11: 452–457.
6. Appassakij H, Silpapojakul K, Wansit R, et al: Evaluation of the immunofluorescent antibody test for the diagnosis of human leptospirosis. Am J Trop Med Hyg 1995; 52: 340.

WASTE MANAGEMENT OR DISPOSABLE:

The contents of RDTs can be divided into :

Infectious waste:

- sharps (lancets, needles, scalpel blades)
- blood collection devices (tubes, straws, and loops); gloves; swabs; and cotton
- used cassettes.

Non-infectious waste (Recyclable):

- packaging materials, desiccant, buffer, and unused or unusable RDTs.

*****You must collect and dispose each type of waste in separate containers as per your waste management policies.***

Index of CE Symbols

Consult instructions for use	For in vitro diagnostic use only	Use by
Catalog #	Lot Number	Tests per kit
Store between 2-30°C	Authorized Representative	Do not reuse
Manufacturer	Date of manufacture	

Mfg. By: BIOGENIX INC. PVT. LTD.
Factory: B - 19/A, S.I.L Ancillary Estate, Amausi Industrial Area, Nadarganj, Kanpur Road,Lucknow - 226008 (U.P.), India
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