One Step IgG/IgM antibody to *Leptospira interrogans* Test

SD BIO LINE LEPTOSPIRA IgG/IgM

Explanation of the test

[INTRODUCTION]

Leptospirosis is a bacterial disease that affects humans and animals. It is caused by bacteria of the genus *Leptospira*. In humans it causes a wide range of symptoms, and some infected persons may have no symptoms at all. Symptoms of leptospirosis include high fever, severe headache, chills, muscle aches, and vomiting, and may include jaundice (yellow skin and eyes), red eyes, abdominal pain, diarrhea, or a rash. If the disease is not treated, the patient could develop kidney damage, meningitis (inflammation of the membrane around the brain and spinal cord), liver failure, and respiratory distress. In rare cases death occurs. Many of these symptoms can be mistaken for other diseases. Leptospirosis is confirmed by laboratory testing of a blood or urine sample. Leptospirosis occurs worldwide but is most common in temperate or tropical climates. It is an occupational hazard for many people who work outdoors or with animals, for example, farmers, sewer workers, veterinarians, fish workers, dairy farmers, or military personnel. It is a recreational hazard for campers or those who participate in outdoor sports in contaminated areas and has been associated with swimming, wading, and whitewater rafting in contaminated lakes and rivers. The incidence is also increasing among urban children.

[INTENDED USE]

The SD BIOLINE LEPTOSPIRA IgG/IgM Test is a solid phase immunochromatographic assay for the qualitative and differential detection of IgG and/or IgM antibody to *Leptospira interrogans* in human serum or plasma. This test is intended for professional use as an aid in the clinical laboratory diagnosis of patients with clinical symptoms consistent with leptospirosis. This test provides only a preliminary test result. Therefore, other serological test like MAT reference test, ELISA, PHA must be used in order to obtain a confirmation of *Leptospira interrogans* infection.

[PRINCIPLE]

The SD BIOLINE LEPTOSPIRA IgG/IgM Test has 3 pre-coated lines, "G" (*Leptospira interrogans* IgG *Test Line*), "M" (*Leptospira interrogans* IgM Test Line) and "C" (Control Line) on the surface of the strip. These lines in result window are not visible before applying any samples. The "Control Line" is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working. A purple "G" or "M" line will be visible in the result window if there are enough IgG and/or IgM antibody to *Leptospira interrogans* in the sample. If IgG and/or IgM antibody to *Leptospira interrogans* are not present in the sample, there is no color appears in "G" or "M" line.

Materials provided/Active ingredients

The SD BIOLINE Leptospira IgG/IgM test kit contains following items to perform the assay.

1. The SD BIOLINE Leptospira IgG/IgM test device : 1 test strip includes
   Gold Conjugates (as main component) : Mouse anti-Leptospira – gold colloid ..... 1 ± 0.2μg
   Antigen Pad (as main component) : Leptospira Lysate ..... 2.2 ± 0.44μg
   Test Line "G" (as main component) : Mouse monoclonal anti-human IgG ..... 5 ±1μg
   Test Line "M" (as main component) : Mouse monoclonal anti-human IgM ..... 5 ±1μg
2. Assay diluent (5ml/bottle)
   100 mM Tri-HCl buffer (as main component)
   Sodium azide (0.02% w/w)
3. Instruction for use

Precaution / kit storage and stability

1. The SD BIOLINE LEPTOSPIRA IgG/IgM Test should be stored at room temperature (1 ~ 30℃)
2. The test device is sensitive to humidity as well as to heat.
3. Perform the test immediately after removing the test device from the container.
4. Do not use it beyond the expiration.
5. DO NOT FREEZE.
6. Do not store the test kit in direct sunlight.

Specimen Collection, Storage and Precaution

1. **[Plasma]**
   Collect the whole blood into the collection tube (containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture and then centrifuge blood to get plasma specimen.
2. **[Serum]**
   Collect the whole blood into the collection tube (not containing anticoagulants such as heparin, EDTA and sodium citrate) by venipuncture, leave to settle for 30 minutes for blood coagulation and then centrifuge blood to get serum specimen of supernatant.
3. If plasma or serum specimens are not tested immediately, they should be refrigerated at 2~8℃ For storage period longer than 2 weeks, freezing is recommended. They should be brought to room temperature (1~30℃) prior to use.
4. Plasma or serum specimens containing a precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

Warnings

1. For *in vitro* diagnostic use only.
2. Do not eat or smoke while handling specimens.
3. Wear protective gloves while handling specimens. Wash hands thoroughly afterwards.
4. Clean up spills thoroughly using an appropriate disinfectant.
5. Decontaminate and dispose of all specimens, reaction kits and potentially contaminated materials, as if they were infectious waste, in a biohazard container.
6. Do not use the test kit if the pouch is damaged or the seal is broken.
7. Specimens and all materials coming into contact with should be handles and disposed of as though potentially infectious.

Procedure of the test

1. Allow all kit components and specimen to room temperature prior to testing.
2. Remove the test device from foil pouch, place it on a flat, dry surface.
3. **[Using a capillary pipette]** With a 5μl capillary pipette, add 5μl of serum or plasma specimen drawn to black line into the square sample well marked "S".
   OR,
   [Using a micropipette] Add 5μl of serum or plasma specimen into the square sample well marked "S".
4. Add 4 drops of assay diluent to the assay diluent well round shaped.
5. Interpret test results in 20 minutes.

Caution : Do not read test results after 20 minutes. Reading too late can give false results.

Interpretation of the test

1. **Negative**
   The control line is only visible on the test device. No IgG and IgM antibodies were detected.
2. **IgM Positive**
   The control line (C) and IgM line (M) are visible on the test device. This is positive for IgM antibodies to Leptospira interrogans.
3. **IgG Positive**
   The control line (C) and IgG line (G) are visible on the test device. This is positive for IgG antibodies to Leptospira interrogans.
4. **IgG and IgM Positive**
   The control line (C), IgM (M) and IgG line (G) are visible on the test device. This is positive for both IgM and IgG antibodies to Leptospira interrogans.
5. **Invalid**
   The control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the likeliest reasons for control line failure. Repeat the test using a new test device.

Limitations of the test

1. This test detects the presence of IgG and IgM antibodies to Leptospira interrogans in the specimen and should not be used as the sole criterion for the diagnosis of leptospirosis.
2. As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
3. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is recommended. Also a negative results does not preclude the possibility of an infection of Leptospira interrogans.

Internal Quality Control

The SD BIOLINE Leptospira IgG/IgM test device has a letter of G, M and C as "Test line 1,2" and "Control line" on the surface of the case. Both the Test Lines and Control line in result window are not visible before applying any samples. The Control Line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working. Control line does not guarantee that the sample has been applied properly, or that the sample was correctly stored particularly.

Expected values

When SD BIOLINE Leptospira IgG/IgM test has been compared with the results confirmed by MAT, the overall accuracy is greater or equal to 90 ± 5%.

Performance characteristics

1. Sensitivity and Specificity
   The SD BIOLINE Leptospira IgG/IgM test have tested with positive and negative clinical samples tested by a leading commercial PHA kit. We used clinical positive and negative specimens confirmed by MAT.
   Table 1. Serological Sensitivity and Specificity of the SD BIOLINE Leptospira IgG/IgM

Sera Characterisation	SD BIOLINE LEPTOSPIRA IgG/IgM			A Commertial PHA		
	Positive	Negative	Total	Positive	Negative	Total
Seropositive (+)	44	1	45	42	3	45
Seronegative (-)	5	95	100	5	95	100
Total	49	96	145	47	98	145

2. Precision

- Within run precision was determined by using 10 replicates of four different specimens containing different concentrations of antibody. The negative and positive values were correctly identified 100% of the time.
- Between run precision was determined by using the four different specimens containing different concentrations of antibody in 3 different replicates with 3 different lots of test devices. Again negative and positive results were observed 100% of the time.

Bibliography of suggested reading

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3. Ryu E. Rapid microscopic agglutination test for Leptospira without nonspecific reaction. Bull Off Int Epizoot. 1970 Jan-Feb;73(1):49-58.
4. Solorzano RF. A comparison of the rapid macroscopic slide agglutination test with the microscopic slide agglutination test for leptospirosis. Proc Annu Meet U S Anim Health Assoc. 1964;68:440-4

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Manufactured by
SD STANDARD DIAGNOSTICS, INC.
156-68 Hagal-dong, Giheung-gu, Yongin-si, Kyonggi-do, Korea
Tel : 82-31-899-2800 Fax : 82-31-899-2840 http://www.standardia.com

CE EC REP Authoried Representative
MT Promedt Consulting GmbH
Altenhofstrasse 80 D-66386 St. Ingbert Germany
Phone : +49 6894 581020, Fax : +49 6894 581021