



Leptospira IgG/IgM Rapid Test Kit (Colloidal Gold)

Instruction for Use

Read this instruction carefully before use.

A rapid test for the qualitative detection of *Leptospira* IgG/IgM in human whole blood, serum and plasma. For professional medical institutions use only, Not for self testing.

PRODUCT NAME

Leptospira IgG/IgM Rapid Test Kit (Colloidal Gold)

SPECIFICATION

25 tests/kit; 5 tests/kit; 1 test/kit

INTENDED USE

The Leptospira IgG/IgM Rapid Test Kit 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 method(s).

SUMMARY AND EXPLANATION THE TEST

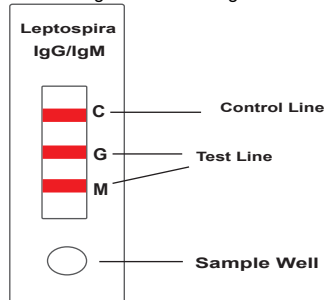
Leptospirosis occurs worldwide and is a common mild to severe health problem for humans and animals, particularly in areas with a hot and humid climate. The natural reservoirs for leptospirosis are rodents as well as a large variety of domesticated mammals. Human infection is caused by L. interrogans, the pathogenic member of the genus of *Leptospira*. The infection is spread via urine from the host animal.

After infection, leptospire 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 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) ELISA; 3) Indirect fluorescent antibody tests (IFATs). However, all above mentioned methods require a sophisticated facility and well-trained technicians.

The 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.

PRINCIPLE

The Leptospira IgG/IgM Rapid Test Kit is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a burgundy colored conjugate pad containing recombinant L. interrogans antigens conjugated with colloid gold (*Leptospira* conjugates) and rabbit IgG-gold conjugates, 2) a nitrocellulose membrane strip containing two test bands (M and G bands) and a control band (C band). The M band is pre-coated with monoclonal anti-human IgM for the detection of anti-L. interrogans IgM, G 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 M 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 G 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 colored band of the immunocomplex of goat anti rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on any of the test bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

REAGENTS AND MATERIALS PROVIDED

Components	25tests/kit	5tests/kit	1test/kit
Cassettes	25 cassettes with dependent sealed foil pouch	5 cassettes with dependent sealed foil pouch	1 cassette with dependent sealed foil pouch
Sample Diluent Solution With Dropper	25tubes (300ul/tube)	5tubes (300ul/tube)	300ul/tube
Transfer tube	25 pcs	5 pcs	1 pcs
Package insert	1	1	1

MATERIALS REQUIRED BUT NOT PROVIDED

Clock or Timer

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 hemolyzed 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 25 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 25 minutes may give erroneous results.
13. Do not perform the test in a room with strong air flow, ie. 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. 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.

SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard bio-safety 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. Separate the plasma by centrifugation.

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.
5. Test specimens as soon as possible after collecting. Store specimens at 2°C to 8°C if not tested immediately.
6. Store specimens at 2°C to 8°C up to 5 days. The specimens should be frozen at -20°C for longer storage

Blood

Drops of whole blood can be obtained by either finger tip puncture or veinpuncture. Do not use any hemolyzed 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. 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.

ASSAY PROCEDURE

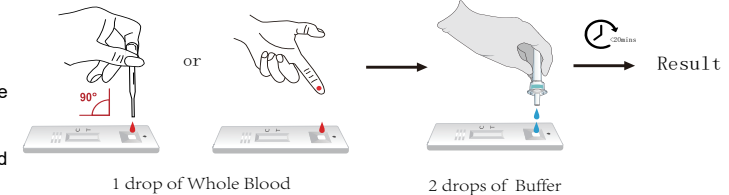
Step 1: Bring the specimen and test components to room temperature if refrigerated or frozen. Once thawed, mix the specimen well prior to assay.

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.

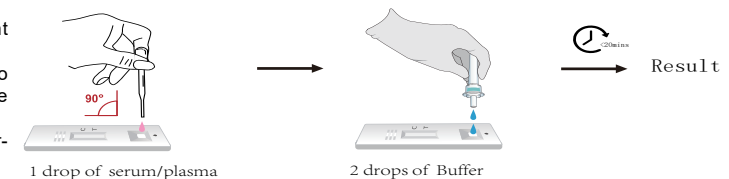
Step 4: For whole blood test

- Apply 1 drop of whole blood (about 20 µL) into the sample well.
- Then add 2 drops (about 60-70 µL) 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 µL-35 µL) of specimen into the sample well making sure that there are no air bubbles.
- Then add 2 drops (about 60-70 µL) of Sample Diluent immediately.



1 drop of serum/plasma

Step 5: Set up timer.

Step 6: Results can be read in 20 minutes. Positive results can be visible in as short as 1 minute.

Don't read results after 30 minutes. To avoid confusion, discard the test device after interpreting the result.

QUALITY CONTROL

Using individual *Leptospira* IgG/IgM Rapid Test Kit 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 test bands (M and G) show no color development.



Positive Control

The C band and two test bands (M and G) show color development.

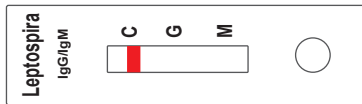


The appearance of any burgundy color in the test bands, regardless of intensity, must be considered as presence of the band.

INTERPRETATION OF ASSAY RESULT

Negative Control

If only the C band is present, the absence of any burgundy color in the both test bands (M and G) indicates that no anti- L.. interrogans antibody is detected. The result is negative.



Positive Control

IgG Positive: 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.



IgM Positive: 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.



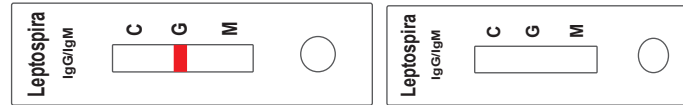
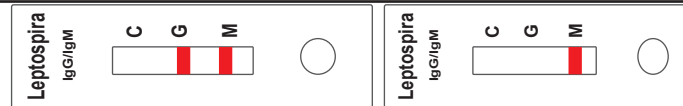
IgG/IgM Positive: In addition to the presence of C band, both M and G bands are developed, the test indicates for the presence of both IgG and IgM anti-Mycoplasma Pneumoniae. The result is also positive.



Samples with positive or reactive results should be confirmed with alternative testing method(s) such as ELISA or PCR and clinical findings before a diagnostic decision is made.

INVALID:

If no C band is developed, the assay is invalid regardless of any burgundy color 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 Rapid Test Kit and by a commercial Leptospira IgM EIA kit. Comparison for all subjects is showed in the following table.

	Leptospira IgG/IgM Combo Rapid Test		
	Positive	Negative	Total
IgM EIA Test			
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 Leptospira IgG/IgM Rapid Test Kit and by a commercial Leptospira IgG EIA kit. Comparison for all subjects is showed in the following table.

	Leptospira IgG/IgM Combo Rapid Test		
	Positive	Negative	Total
IgG EIA			
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

- 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.
- The Leptospira IgG/IgM Rapid Test Kit is limited to the qualitative detection of antibodies to L.. interrogans in human serum, plasma or whole blood. The intensity of the test band does not have linear correlation with antibody titer in the specimen.
- 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.
- 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.
- Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
- The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

SYMBOLS

Symbol	Used For	Symbol	Used For
	Use-by date		Consult instructions for use
LOT	Batch code	IVD	In vitro diagnostic medical device
	Temperature limit		Manufacturer
	Please don't reuse it		Keep away from sunlight
	Don't use the product when the package is damaged		Keep dry
	Date of manufacture		Tests per kit
CE	CE Mark		Biological Risks
EC REP	Authorized representative in the European Community		

BASIC INFORMATION



Ningbo BESTest Bio-technology Co.,Ltd.

Address: No.80 building, No.777, Qing Feng Road, Cicheng Town, Jiangbei District, Ning Bo, Zhejiang, China 315033
Tel: 0086 571 2799 8736



SUNGO Europe B.V.

Address: Olympisch Stadion 24, 1076DE Amsterdam, Netherlands.