OnSite® Leptospira IgG/IgM Combo Rapid Test

REF R0101C

Instructions for Use

INTENDED USE

The *OnSite* Leptospira IgG/IgM Combo 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 by professionals as 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

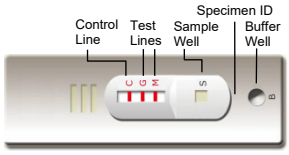
Leptospirosis occurs worldwide and is a common mild to severe health problem for humans and animals, particularly in areas with hot and humid climates. The natural reservoirs for leptospirosis are rodents as well as a large variety of domesticated mammals. Human infection is caused by *L. interrogans*, the pathogenic member of the genus of *Leptospira*[1,2]. 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. Antibodies to leptospira are detectable by the 6th to 10th day of disease and levels peak within 3 to 4 weeks and then gradually decline[3]. Antibodies may be detectable for years post infection[3]. IgM anti-leptospira are detectable during the first week of illness allowing early therapeutic intervention at a time point where it is most effective[3]. IgG class antibodies appear at a later time point of infection and may be 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)[4]; 2) ELISA[5,6]; 3) Indirect fluorescent antibody tests (IFATs)[6]. 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 early stage of infection in the 1st and 2nd weeks after exposure and leptospiremia ends by the first week of illness[3]. All above mentioned methods require a sophisticated facility and well-trained technicians.

The *OnSite* Leptospira IgG/IgM Combo Rapid Test is a simple serological test that utilizes antigens from *L. interrogans* to detect and discriminate IgG and IgM antibodies to leptospira. The test can be performed within 15-20 minutes by minimally skilled personnel without the use of laboratory equipment.

TEST PRINCIPLE



The *OnSite* Leptospira IgG/IgM Combo Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a 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 M 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* IgM, 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 colored M line, indicating a anti-*L. interrogans* IgM positive test result. 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 colored G line, indicating a 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 should exhibit a 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 retested with another device.

REAGENTS AND MATERIALS PROVIDED

1. Individually sealed foil pouches containing:
   a. One cassette device
   b. One desiccant
2. 5 µL capillary tubes
3. Sample diluent (REF SB-R0101, 5 mL/bottle)
4. Instructions for Use

MATERIALS MAY BE REQUIRED BUT NOT PROVIDED

1. Positive control
2. Negative control

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. Read these instructions for use completely before performing the test. Failure to follow the instructions could lead to 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 (15-30°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 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 the kit 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.

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.

Test specimens as soon as possible after collecting. Store specimens at 2-8°C, if not tested immediately. The specimens can be stored at 2-8°C 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 lipemia, gross hemolysis or turbidity in order to avoid interference with result interpretation.

Whole Blood

Step 1: Drops of whole blood can be obtained by either fingertip puncture or 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

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

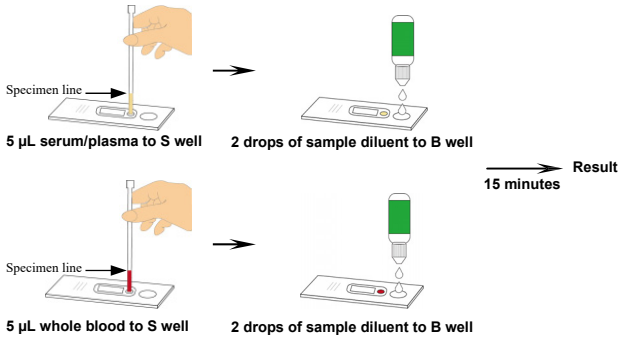
Step 2: When ready to test, open the pouch at the notch and remove the device. Place the device on a clean, flat surface.

Step 3: Be sure to label the device with the specimen's ID number.

Step 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.***

Holding the capillary tube vertically, dispense the entire specimen into the center of the sample 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.



5 µL serum/plasma to S well **2 drops of sample diluent to B well**

5 µL whole blood to S well **2 drops of sample diluent to B well**

Step 5: Set up timer.

Step 6: 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 color in both test lines (M and G) indicates that no detectable anti-*L. interrogans* antibody is present in the specimen. The result is negative or non-reactive.

2. **POSITIVE RESULT**:

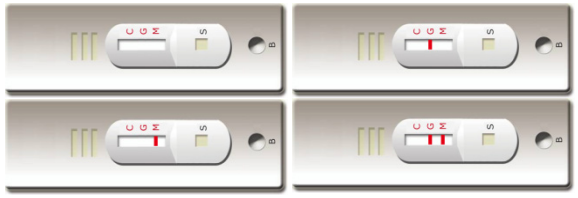
2.1 In addition to the presence of the C line, if only the M line develops, the test indicates the presence of anti-*L. interrogans* IgM. The result is anti-*L. interrogans* IgM positive or reactive.

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 color in the test lines as indicated below. Repeat the assay with a new device.



PERFORMANCE CHARACTERISTICS

1. **Accuracy**

Specimens collected from suspicious patients and normal individuals were studied. The *OnSite* Leptospira IgG/IgM Combo Rapid Test shows 100% specificity (95% CI: 47.8-100%) and 100% sensitivity (95% CI: 78.2-100%) 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 *OnSite* Leptospira IgG/IgM Combo Rapid Test according to the standard procedure. The results showed that the following specimens (n=3-10) did not cross-react with the *OnSite* Leptospira IgG/IgM Combo Rapid Test.

HAV	HBV	HCV	HEV	*H. pylori*
hCG	HIV	Dengue	TB	*T. pallidum*
Typhoid	ANA	HAMA	RF (up to 8,400 IU/mL)	

3. **Interference**

Common substances (such as pain and fever medication, blood components) may affect the performance of the *OnSite* Leptospira IgG/IgM Combo Rapid Test. This was studied by spiking these substances into negative and positive standard controls. The results are presented in the following table and demonstrate, at the concentrations tested, the substances studied do not affect the performance of the *OnSite* Leptospira IgG/IgM Combo Rapid Test.

List of potentially interfering substances and concentrations tested:

1. Albumin	60 g/L	6. Hemoglobin	2 g/L
2. Bilirubin	20 mg/dL	7. Heparin	3,000 U/L
3. Creatinine	442 µmol/L	8. Human IgG	1000 mg/dL
4. EDTA	3.4 µmol/L	9. Salicylic acid	4.34 mmol/L
5. Glucose	55 mmol/L	10. Sodium Citrate	3.8%

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation Assay Result sections must be followed closely when testing for the presence of IgG and IgM antibodies to pathogenic *L. interrogans* in human serum, plasma or whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *OnSite* Leptospira IgG/IgM Combo Rapid Test is limited to the qualitative detection of IgG and IgM 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 anti-*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 anti-*L. interrogans* IgG and IgM 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. Infection may progress rapidly. If the symptom persists, while the result from *OnSite* Leptospira IgG/IgM Combo Rapid Test is negative or non-reactive, it is recommended to test with an alternative test method.
6. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor may affect expected results.
7. Depending on the circulating Leptospira serovars regionally present at the time of collection, sensitivity of this product may vary.
8. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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2. Levett PN. Leptospirosis. Clin Microbiol Rev 2001;14:296-326
3. Ahmad SN, Shah S, Ahmad FMH. Laboratory diagnosis of Leptospirosis. J Postgrad Med 2005;51:195-200.
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6. 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.
7. 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.

Index of Symbols

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

CTK Biotech, Inc.
13855 Stowe Drive
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

PI-R0101C Rev. F2.0
Date released: 2020-11-12
English Version

For Export Only, Not For Re-sale in the USA