todylaboratories®

Rapid Tests

CE IVD REF C61RT1009

Leptospira IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) Package Insert

A rapid test for the qualitative detection of IgG and IgM antibodies to Leptospira interrogans in human's whole blood, serum or plasma specimen.
For professional in vitro diagnostic use only.

INTENDED USE

The Leptospira IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies to Leptospira interrogans in human's whole blood, serum or plasma.

SUMMARY

Leptospirosis occurs worldwide and is a common mild to severe health problem for humans and animals, particularly in area 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 Leptospira interrogans, the pathogenic member of the genus of Leprospira[1,2]. The infection is spread via urine from the host animal.
After infection, leptospires are present in the blood until they are cleared after 4 to 7 days following the production of anti-Leptospira 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- Leptospira 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)[6]. However, all above mentioned methods require a sophisticated facility and well-trained technicians.

PRINCIPLE

The Leptospira IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a qualitative, membrane based immunoassay for the detection of IgG and IgM antibodies to Leptospira in whole blood, serum or plasma. The membrane is pre-coated with recombinant mouse anti- human IgG and mouse anti-human IgM on the test line region of the cassette. During testing, the whole blood, serum or plasma specimen reacts with recombinant Leptospira interrogans antigen conjugated colloid gold. The mixture migrates upward on the membrane chromatographically by capillary action to react with mouse anti-human IgG or/and mouse anti-human IgM on the membrane and generate a colored line. Presence of this colored line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

REAGENTS

The test cassette contains recombinant Leptospira interrogans antigen conjugated colloid gold, mouse anti-human IgG and mouse anti-human IgM coated on the membrane.

PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
- Humidity and temperature can adversely affect results.

STORAGE AND STABILITY

The kit can be stored at room temperature or refrigerated (2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. **DO NOT FREEZE**. Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

- The Leptospira IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) can be performed using whole blood (from venipuncture or fingerstick), serum or plasma.
- To collect Fingerstick Whole Blood specimens:
  - Wash the patient's hand with soap and warm water or clean with an alcohol swab. Allow to dry.
  - Massage the hand without touching the puncture site by rubbing down the hand towards the fingertip of the middle or ring finger.
  - Puncture the skin with a sterile lancet. Wipe away the first sign of blood.
  - Gently rub the hand from wrist to palm to finger to form a rounded drop of blood over the puncture site.
  - Add the Fingerstick Whole Blood specimen to the test cassette by using a capillary tube:
    - Touch the end of the capillary tube to the blood until filled to approximately 40 µL. Avoid air bubbles.
    - Place the bulb onto the top end of the capillary tube, then squeeze the bulb to dispense the whole blood to the specimen well of the test cassette.
  - Add the Fingerstick Whole Blood specimen to the test cassette by using hanging drops:
    - Position the patient's finger so that the drop of blood is just above the specimen well of the test cassette.
    - Allow 1 hanging drop of fingerstick whole blood to fall into the center of the specimen well on the test cassette, or move the patient's finger so that the hanging drop touches the center of the specimen well. Avoid touching the finger directly to the specimen well.
- Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, non-hemolyzed specimens can be used.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days.For long term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2- 8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with federal regulations for transportation of etiologic agents.

MATERIALS

Materials provided

• Test Cassettes • Droppers • Package Insert • Buffer

Materials required but not provided

• Specimen Collection Containers • Lancets (for fingerstick whole blood only) • Centrifuge (for plasma only) • Timer • Heparinized capillary tubes and dispensing bulb (for fingerstick whole blood only)

DIRECTIONS FOR USE

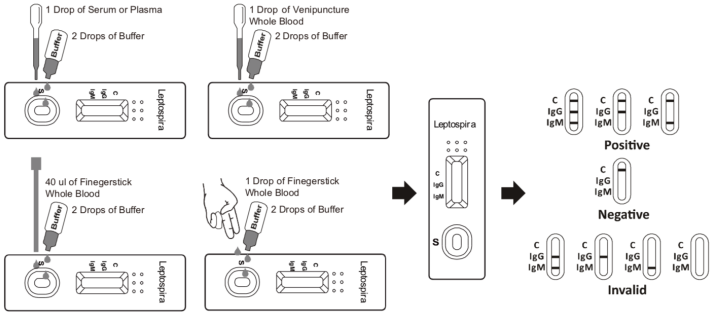
Allow test cassette, specimen, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
2. Place the cassette on a clean and level surface.

For **Serum or Plasma** specimen: Hold the dropper vertically and **transfer 1 drop of serum or plasma** (approximately 40 µL) to the specimen well, then **add 2 drops of buffer** (approximately 80 µL),and start the timer, see illustration below.
For **Venipuncture Whole Blood** specimen: Hold the dropper vertically and transfer **1 drop of whole blood** (approximately 40 µL) to the specimen well, then **add 2 drops of buffer** (approximately 80µL), and start the timer. See illustration below.
For **Fingerstick Whole Blood** specimen:

- To use a capillary tube: Fill the capillary tube and **transfer approximately 40 µL of fingerstick whole blood specimen** to the specimen well of test cassette, then **add 2 drops of buffer** (approximately 80 µL) and start the timer. See illustration below.
- To use hanging drops: Allow **1 hanging drop of fingerstick whole blood specimen** (approximately 40 µL) to fall into the specimen well of test cassette, then **add 2 drops of buffer** (approximately 80 µL) and start the timer. See illustration below.

3. Wait for the colored line(s) to appear. Read the results at 15 minutes, do not interpret the results after 20 minutes.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

IgG POSITIVE: Two distinct colored lines appear. One colored line should be in the control region (C) and another colored line should be in the IgG region.

IgM POSITIVE: Two distinct colored lines appear. One colored line should be in the control region (C) and another colored line should be in the IgM region.

IgG and IgM POSITIVE: Three distinct colored lines appear. One colored line should be in the control region (C) and another two colored lines should be in the IgG and IgM regions. NOTE: The intensity of the color in the IgG and IgM region(s) will vary depending on the concentration of Leptospira antibodies present in the specimen. Therefore, any shade of red in the IgG and IgM region(s) should be considered positive.

NEGATIVE: One colored line appears in the control region (C). No apparent red or pink line appears in the IgG and IgM region(s).

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

Internal procedural controls are included in the test. A colored line appearing in the control region (C) is an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

LIMITATIONS

1.The Direction for Use and the Interpretation of Result must be 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.
2.The Leptospira IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is limited to the qualitative detection of antibodies to Leptospira 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.
3.A negative result for an individual subject indicates absence of detectable Leptospira interrogans antibodies. However, a negative test result does not preclude the possibility of exposure to Leptospira interrogans.
4.A negative result can occur if the quantity of Leptospira 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 titer 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.

EXPECTED VALUES

The Leptospira IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) has been compared with a leading commercial Leptospira IgG ELISA, Leptospira IgM ELISA test. The correlation between these two systems is over 98%.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

A total of 230 samples from susceptible subjects were tested by the Leptospira IgG/IgM Rapid Test Cassette and by a commercial Leptospira IgM ELISA kit. Comparison for all subjects is shown in the following table.

IgM Results

Method		ELISA		Total Results
Leptospira IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	9	3	12
	Negative	1	217	218
Total Results		10	220	230

Relative Sensitivity: 90.0% (95%CI*: 55.5%-99.7%)
Relative Specificity: 98.6% (95%CI*: 96.1%-99.7%)
Accuracy: 98.3% (95%CI*: 94.7%-98.9%) *Confidence Intervals

A total of 239 samples from susceptible subjects were tested by the Leptospira IgG/IgM Rapid Test Cassette and by a commercial Leptospira IgG ELISA kit. Comparison for all subjects is shown in the following table.

IgG Results

Method		ELISA		Total Results
Leptospira IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	15	3	18
	Negative	1	220	221
Total Results		16	223	239

Relative Sensitivity: 93.8% (95%CI*: 69.8%-99.8%)
Relative Specificity: 98.7% (95%CI*: 96.1%-99.7%)
Accuracy: 98.3% (95%CI*: 95.8%-99.5%) *Confidence Intervals

Precision

Intra-Assay

Within-run precision has been determined by using 20 replicates of five specimens: a negative, a Leptospira IgM low titer positive, a Leptospira IgM high titer positive, a Leptospira IgG low titer positive and a Leptospira IgG high titer positive. The negative, a Leptospira IgM low titer positive, a Leptospira IgM high titer positive, a Leptospira IgG low titer positive and a Leptospira IgG high titer positive values were correctly identified 100% of the time.

Inter-Assay

Between-run precision has been determined by 20 independent assays on the same five specimens: a negative, a Leptospira IgM low titer positive, a Leptospira IgM high titer positive, a Leptospira IgG low titer positive and a Leptospira IgG high titer positive. Three different lots of the Leptospira IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) have been tested over a 3-Days period using negative, a Leptospira IgM low titer positive, a Leptospira IgM high titer positive, a Leptospira IgG low titer positive and a Leptospira IgG high titer positive specimens. The specimens were correctly identified 100% of the time.

Cross-reactivity

The Leptospira IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) has been tested by HAMA, RF, HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, Syphilis, HIV, H. Pylori, MONO, CMV, Rubella and TOXO positive specimens. The results showed no cross-reactivity.

Interfering Substances

The following potentially interfering substances were added to Leptospira interrogans negative and positive specimens. No interference observed.

Acetaminophen: 20 mg/dL	Acetylsalicylic Acid: 20 mg/dL	Ascorbic Acid: 2g/dL
Caffeine: 20 mg/dL	Gentisic Acid: 20 mg/dL	Albumin: 2 g/dL
Creatin: 200 mg/dL	Bilirubin: 1g/dL	Hemoglobin: 1000mg/dL
Oxalic Acid: 60mg/dL		

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