LEPTOSPIRA CARD

For *in Vitro* diagnostic use only

Immunochromatographic test CARD for qualitative detection of IgG/IgM antibody to Leptospira in human serum, plasma and whoole blood

I. INTENDED USE

The Leptospira Card 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 Card test must be confirmed with alternative testing method(s).

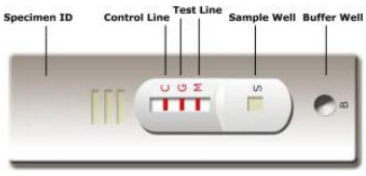
II. SUMMARY AND EXPLANATION OF THE TEST

Leptospirosis occurs worldwide and is a common mild to severe health problem for humans and animals, particularly in areas with 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[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-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)[3]; 2) ELISA[4-5]; 3) Indirect fluorescent antibody tests (IFATs)[6]. However, all above mentioned methods require a sophisticated facility and well-trained technicians.

The Leptospira Card 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.

III. TEST PRINCIPLE



The Leptospira Card 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 T 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 T bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

IV. REAGENTS AND MATERIALS PROVIDED

- Each kit contains 10 test devices, each in a foil pouch with two items inside:
  - One cassette device
  - One dessicant.
- Sample diluent (1 bottle, 1.5 ml)
- 5 µL capillary tubes (10 items)
- Instructions for use

V. MATERIALS REQUIRED BUT NOT PROVIDE

- Clock or timer
- Lancing device for whole blood test.

VI. PRECAUTIONS

1. For professional and in vitro diagnostic use.
2. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
3. Do not open the sealed pouch, unless ready to conduct the assay.
4. Do not use expired devices.
5. Bring all reagents to room temperature (15°C-30°C) before use.
6. Do not use the components in any other type of test kit as a substitute for the components in this kit.
7. Do not use hemolized blood specimen 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. 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. The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 15 minutes may give erroneous results.
12. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.

VII. 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 over 30°C.

VIII. SPECIMEN COLLECTION AND HANDLING

Consider any materials of human origin as infectious and handle them using standard biosafety 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.

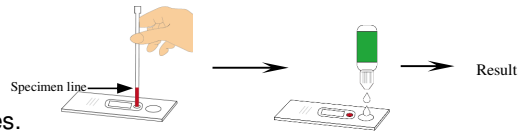
Test specimens as soon as possible after collecting. Store specimens at 2°C-8°C if not tested immediately.
Store specimens at 2°C -8°C up to 5 days. The specimens should be frozen at -20°C for longer storage.
Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen specimens to room temperature 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.

Whole blood

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

IX. ASSAY PROCEDURE

1. Allow samples and reagents to come to room temperature prior to testing if refrigerated or frozen. Mix the specimen well prior to assay once thawed.
2. Remove the «reaction device» from its protective wrapper. Place the test device on a clean, flat surface.
3. Label device with the patient's name or control number.
4. Fill in the capillary with the specimen not to exceed the specimen line as showed in the following image (about 5 µL). Holding the dropper vertically, dispense all of the specimen into the center of the sample well (S) making sure that there are no air bubbles.



Note: Practice a few times prior to testing if you are not familiar with the mini dropper. For better precision, transfer specimen by pipette capable to deliver 5µL of volume.

5. Add 2 drops (60-80 µl) of Diluent sample immediately in Buffer well (B).
6. Read the test result in 15 to 20 min. 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.

X. INTERPRETATION OF ASSAY RESULT

1. NEGATIVE RESULT If only the C band is present, the absence of any burgundy color in the both T bands (M and G) indicates that no antibodies IgM e IgG anti- *L. interrogans* are detected. The result is negative.

2.1 POSITIVE RESULT: In addition to the presence of C band, if only M band is developed, indicates for the presence of IgM anti-L.*interrogans*; the result is positive.

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

In addition to the presence of C band, both M and G bands are developed, indicates for the presence of 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 band is developed, the assay is invalid regardless of any burgundy color in the M and G bands as indicated below. Repeat the assay with a new device.

XI. PERFORMANCE CHARACTERISTICHS

Accuracy

Specimens collected from suspicious patients and normal individuals were studied. The Leptospira Card 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.

Cross reactivity

Specimens from other infectious diseases were tested for creoss-reactivity with the Leptospira Card according to the standard procedure. The results showed that the following specimens (n=3-10) did not cross-react with Leptospira Card.

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

Intereference

Common substances (such as pain and fever medication, blood components) may affect the performance of Leptospira Card. 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 Leptospira Card.

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

XII. LIMITATIONS OF TEST

1. 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.
2. The Leptospira Card 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.
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 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.

XIII. REFERENCES

1. Stallman GND. The International Committee on Systematic Bacteriology: Sub committee 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

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (EXXX)		Manufacturer		Keep dry	NON STERILE	Non-sterile
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

CONTENT (10 tests)
Leptospira Card
Sample diluent
5 µL capillary
Instruction for use

COD. VQ85100
10 devices
1 x 1.5 mL
10 items
1 item

EDMA Code 15 05 10 90 00 CE IVD