PreventID® **Leptospira**

Manual (FOR PROFESSIONAL USE)

→REF KSTILEP402G10 | KSTILEP402GP

Intended Use

PreventID® Leptospira is a rapid chromatographic immunoassay for the qualitative detection of IgG and IgM antibodies to *Leptospira interrogans* in human whole blood, serum or plasma specimen.

Introduction

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 *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 first to second 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.

Test Principle

The **PreventID® Leptospira** rapid test 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 coloured line. Presence of this coloured line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a coloured line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred. The test device contains recombinant *Leptospira interrogans* antigen conjugated colloid gold, mouse anti-human IgG and mouse anti-human IgM coated on the membrane.

Materials

Materials provided

- test devices (with sample droppers), individually packed TEST
- buffer BUF
- manual

Materials Required but not Provided: timer or stop watch, gloves, blood specimen collection containers; lancets and heparinized capillary tubes and dispensing bulb (for finger tip blood); centrifuge for serum or plasma

Storage and Stability

Store the test between 2°C and 30°C; **do not freeze**. The test device is sensitive to humidity as well as to heat. Perform the test immediately after removing the test device from the pouch. Do not use it beyond the expiry date.

Precautions

1. For in vitro diagnostic use only.
2. Do not eat or smoke while handling specimen.
3. Wear protective gloves and wash hands thoroughly after performing the test.
4. Avoid splashing or aerosol formation while handling specimen and performing the test.
5. All samples and materials used should be treated as potentially infectious and disposed in a biohazard container. Clean all contaminated objects and surfaces carefully. The used test should be discarded according to local regulations.
6. Do not use test if the pouch is torn or if the membrane of the test device is visibly damaged. Note the expiry date.
7. Read the instruction carefully before performing the test.
8. Do not mix reagents from different lots.
9. If you have any questions please contact Preventis GmbH.

Note: The same lancet should only be used for one person and should not be shared with another person, because the used lancet is biohazard.

Sample Collection and Sample Preparation

PreventID® Leptospira can be performed using whole blood (from venipuncture or fingertip), serum or plasma.

Fingertip whole blood specimens:

- Wash the patient's hand with soap and warm water and 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 fingertip whole blood specimen to the test device 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 sample application window of the test device.

→ Add the fingertip whole blood specimen to the test by using **hanging drops:**

- Position the patient's finger so that the drop of blood is just above the sample application window of the test device.
- Allow 1 hanging drop of fingertip whole blood to fall into the center of the sample application window on the test device, or move the patient's finger so that the hanging drop touches the center of the sample application window. Avoid touching the finger directly to the sample application window.

Serum and plasma specimens:

- Separate serum or plasma from blood as soon as possible to avoid haemolysis. Only clear, non-haemolysed 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 fingertip 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.

Test Procedure

Allow the test, specimen, buffer and/or controls to reach room temperature (15–30°C) prior to testing.

1. Remove the test device from its pouch, place it on a flat dry surface and use it as soon as possible. Best results will be obtained if the assay is performed within one hour. The oval sample application window at the one end of the test device should be at the right side.
2. For serum or plasma specimen:
   Hold the dropper vertically and transfer **1 drop of serum or plasma** (approximately 40 µL) to the sample application window, then **add 2 drops of buffer** (approximately 80 µL),and start the timer *(Fig. 1a)*.
   For venipuncture whole blood specimen:
   Hold the dropper vertically and transfer **1 drop of whole blood** (approximately 40 µL) to the sample application window, then add 2 drops of buffer (approximately 80 µL), and start the timer *(Fig. 1b)*.
   For fingertip whole blood specimen:
   To use a capillary tube: Fill the capillary tube and transfer approximately **40 µL of fingertip whole blood specimen** to the sample application window of the test device, then add **2 drops of buffer** (approximately 80 µL) and start the timer *(Fig. 1c)*.
   To use hanging drops: Allow **1 hanging drop of fingertip whole blood specimen** (approximately 40 µL) to fall into the sample application window of test device, then add **2 drops of buffer** (approximately 80 µL) and start the timer*(Fig. 1d)*.
3. Wait for the coloured line(s) to appear. Read the results at **15 minutes**, do not interpret the results after 20 minutes.

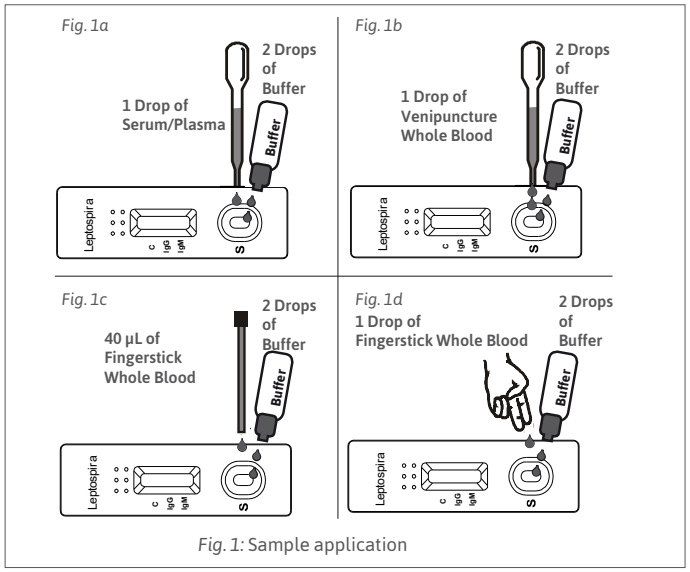

Fig. 1: Sample application

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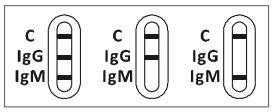
Test Interpretation

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

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

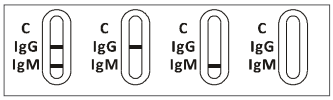
IgG and IgM Positive: Three distinct coloured lines appear. One coloured line should be in the control region (C) and another two coloured lines should be in the IgG and IgM regions.



Note: The intensity of the colour 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 coloured line appears in the control line region (C). No 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. If the problem persists, discontinue using the test kit immediately and contact Preventis.



Caution: In order to prevent incorrect results the test should not be interpreted later than after 20 minutes.

Test Characteristics

Expected Values

The **PreventID® Leptospira** rapid test has been compared with a leading commercial Leptospira IgG ELISA, Leptospira IgM ELISA test. The correlation between these two systems is over 98%.

Sensitivity and Specificity

A total of 230 samples from susceptible subjects were tested by the **PreventID® Leptospira** test and by a commercial Leptospira IgM ELISA kit. Comparison for all subjects is shown in the following table.

IgM Results:		ELISA		
PreventID® Leptospira	Results	Postive	Negative	**Total Result**
	Positive	9	3	12
	Negative	1	217	218
Total Result		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 **PreventID® Leptospira** and by a commercial Leptospira IgG ELISA kit. Comparison for all subjects is shown in the following table.

IgG Results:		ELISA		
PreventID® Leptospira	Results	Postive	Negative	**Total Result**
	Positive	15	3	18
	Negative	1	220	221
Total Result		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

Reproducibility

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 **PreventID® Leptospira** test 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 **PreventID® Leptospira** test 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.

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

None of the substances at the concentration tested interfered in the assay.

Quality Control

Internal procedural controls are included in the test. A coloured 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.

Test Limitations

1. The test procedure and test interpretation must be followed carefully 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 **PreventID® Leptospira** test is limited to the qualitative detection of antibodies to *Leptospira interrogans* in human serum, plasma or whole blood. The intensity of the test line 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 heterogeneous 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.

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.

US: all products: Research Use Only. Not for use in diagnostic procedures.

Temperature limitation	Manufacturer
In vitro diagnostic device	Lot number
Catalogue number	Expiry date
To be used with	Do not reuse
Read user instructions	Contains sufficient for <n> tests
Keep away from sunlight	

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Status: 2019-11-08

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