SBio Lepto Test

Rapid test for IgM antibodies to Leptospira

REF	90221010
Σ	10 T

CE

Temperature Limitation	Manufacturer	PIPETTE — Disposable Plastic Sample Applicator	EC REP — Authorised Representative in the European Community	Xn NaN$_3$ R22 S23-46-61 Harmful if swallowed. Do not breathe vapour. If swallowed, seek medical advice immediately and show this container or label. Avoid release to the environment. Refer to special instructions.
Use by (Last day of stated month)	Consult Instructions for use	DEVICE — Device	BUF — Sample Running Buffer	
Date of Manufacture	REF — Catalogue Number	Σ — Contains sufficient for <n> tests	Do not reuse	
LOT — Batch Number/ Lot Number	IVD — *In vitro* Diagnostic Medical Device	This side up	Lepto — Rapid test for IgM antibodies to Leptospira	

INTENDED USE

SBio Lepto Test is a rapid, qualitative, sandwich immunoassay for the detection of Leptospira specific IgM antibodies in human serum/plasma or whole blood specimen. It is useful for the serodiagnosis of current or recent Leptospirosis. The broadly reactive genus specific antigen employed in the test allows the detection of Leptospira infections caused by a wide range of strains of different serovars.

SUMMARY

Leptospira are actively motile, delicate spirochaetes possessing a large number of closely wound spirals and characteristic hooked ends. There are several species of Leptospira and they may be saprophytic or parasitic. They can be distinguished only under dark ground illumination in the living state or by electron microscopy. Leptospirosis is a zoonotic disease of worldwide prevalence. Humans are infected when the water contaminated by the urine of carrier animals enters the body through cuts or abrasions on the skin or through intact mucosa of the mouth, nose or conjunctiva. Clinical symptoms include fever, chills, headache, conjunctivitis, myalgia and GI related symptoms, Kidney infection is a common sequelae.

Diagnosis may be made by demonstration of Leptospires microscopically in blood or urine, by isolating them in culture or by inoculation of guinea pigs or by serological tests.

SBio Lepto Test, qualitatively detects the presence of IgM class of Leptospira specific antibodies in human serum/plasma or whole blood specimen.

PRINCIPLE

SBio Lepto Test utilizes the principle of agglutination of antibodies/ antisera with respective antigen in immuno-chromatography format along with use of nano gold particles as agglutination revealing agent. As the test sample flows through the membrane assembly of the test device, the Agglutinating sera for Human IgM - colloidal gold conjugate forms a complex with IgM antibodies in the sample. This complex moves further on the membrane to the test window 'T' where it is immobilized by the broadly reactive Leptospira genus specific antigens coated on the membrane, leading to the formation of a red to deep purple coloured band at the test region 'T' which confirms a positive test result. Absence of this coloured band in test region 'T' indicates a negative test result. The unreacted conjugate and the unbound complex if any, along with rabbit globulin-colloidal gold conjugate move further on the membrane and are subsequently immobilized by the Agglutinating sera for rabbit globulin coated at the control region 'C' of the membrane assembly, forming a red to deep purple coloured band. The control band serves to validate the test results.

REAGENTS AND MATERIALS SUPPLIED

SBio Lepto Test kit contains:

A. Individual pouches, each containing:
   1. Test Device: Membrane test assembly pre-dispensed with the Agglutinating sera for Human IgM - colloidal gold conjugate, Rabbit globulin-colloidal gold conjugate, Leptospira genus specific antigens at test window 'T' and Agglutinating sera for rabbit globulin pre-dispensed at the control window 'C'.
   2. Desiccant pouch.
   3. Disposable Plastic Sample Applicator.

B. Sample Running Buffer in a dropper bottle.

C. Package Insert.

STORAGE AND STABILITY

The sealed pouches in the test kit & the kit components may be stored between 4^{0}C to 30^{0}C till the duration of the shelf life as indicated on the pouch/carton. DO NOT FREEZE. After first opening of the sample running buffer bottle, it can be stored between 4^{0}C to 30^{0}C for remaining duration of its shelf life.

OPTIONAL MATERIAL REQUIRED

Calibrated micropipette capable of delivering 10µl sample accurately.

NOTES

1. Read the instructions carefully before performing the test.
2. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use only.
3. Do not use the kit beyond expiry date.
4. Do not reuse the test device.
5. Do not intermix the reagents from different lots.
6. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm.
7. Handle all specimens as potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infective material.
8. Sample Running Buffer contains Sodium Azide (0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build-up in the plumbing.

SPECIMEN COLLECTION AND PREPARATION

1. Blood samples collected with a suitable anticoagulant such as EDTA or Heparin or Oxalate can also be used.
2. No prior preparation of the patient is required before sample collection by approved techniques.
3. Fresh serum / plasma is preferable. Anticoagulated whole blood can also be used as specimen. Serum / plasma may be stored at 2^{0}C-8^{0}C up to 24 hours in case of delay in testing. For long-term storage, freeze the specimen at -20^{0}C for 3 months or -70^{0}C for longer periods. Whole blood should be used immediately and should not be frozen.
4. Repeated freezing and thawing of the specimen should be avoided.
5. Do not use haemolysed, clotted, contaminated, viscous/turbid specimen.
6. Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only should be used for testing.
7. For each sample, a new sample applicator should be used.

TESTING PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the SBio Lepto Test kit components to room temperature before testing.
2. Open the pouch and retrieve the device, sample applicator and desiccant pouch. Check the color of the desiccant. It should be blue, if it has turned colorless or pink, discard the device and use another device. ***Once opened, the device must be used immediately.***
3. Label the test device with patient's identity.
4. Tighten the vial cap of the sample running buffer bottle provided with the kit in the clockwise direction to pierce the dropper bottle nozzle.
5. Add 10µl of serum/ plasma or whole blood with a micropipette into the sample port 'A', OR using the 5µl sample applicator provided with the kit, dip the applicator into the sample and then blot into the sample port 'A'. Repeat this step twice for each sample. Ensure that the applicator does not retrieve clots or debris from the sample.
6. Immediately dispense 5 drops of sample running buffer in the buffer port 'B', by holding the plastic dropper bottle vertically.
7. At the end of **15 minutes,** read the results as follows:

Negative Result:
Only one coloured band appears in the control window 'C'.

Positive Result:
In addition to the control band, another red to deep purple coloured band appears in the test window 'T'.

Invalid Result:
The test should be considered invalid if no bands appear on the device. The test should also be considered invalid if only test band appears and no control band appears. Repeat the test with a new device ensuring that the test procedure has been followed accurately.

PERFORMANCE CHARACTERISTICS

Internal Evaluation

In an in-house study, the performance of SBio Lepto Test was evaluated using a panel of 70 negative specimens and 20 positive specimens for Leptospirosis infection whose results were earlier confirmed with a commercial Leptospira IgM ELISA kit. The results of the evaluation are as follows:

Specimen data	No. of samples tested	SBio Lepto Test	Commercial ELISA
No. of Positive specimens	20	19	20
No. of Negative specimens	70	67	70

Based on this evaluation, Sensitivity of SBio Lepto Test : 95%
Specificity of SBio Lepto Test : 95.7%.

External Evaluation

SBio Lepto Test was evaluated at the New Civil Hospital, Surat, India, in parallel with two commercially available rapid screening tests for leptospirosis and their results were compared with the gold standard test, the Leptospira IgM ELISA test. Total 100 samples were evaluated, 80 were IgM ELISA positive and 20 were IgM ELISA negative. The results of the study was as follows:

Screening test	% Sensitivity	% Specificity
SBio Lepto Test	90.7	93.4
Commercial Rapid (Latex based)	89.7	90.9
Commercial Rapid test	53.7	60

LIMITATIONS OF THE TEST

(1) The intensity of the test line depends upon the stage of the disease and the titres of the antibodies in the test specimen. (2) As specific antibodies reach detectable levels about one week after the onset of disease, a sample collected very early may yield a negative test result. (3) If the test is negative and if Leptospirosis is still suspected, the test should be repeated with the second sample collected at a later date in conjunction with clinical reexamination. (4) In endemic areas faint bands may appear occasionally due to borderline IgM titres present as a result of previous exposures. (5) It is recommended that the positive results obtained must be reconfirmed using a confirmatory test such as the MAT (Microscopic agglutination test). (6) High titres of RF and heterophile antibodies may interfere with the test, in such cases the results must be interpreted with caution. (7) The results must be correlated with clinical findings to arrive at the diagnosis. (8) Do not interpret the test result beyond 30 minutes.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

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