Test-it™ Leptospira IgM Lateral Flow Assay
Leptospira-specific immunoassay for use with human serum or whole blood samples

Product Code
IgM: LEP001

Intended use
Life Assay manufactures the Test-it™ Leptospira IgM lateral flow assay for the rapid serodiagnosis of leptospirosis. The test is aimed at the detection of disease specific IgM antibodies in human serum or whole blood samples.

Introduction
Leptospirosis or Weil’s disease is caused by pathogenic spirochetes belonging to the genus Leptospira. Leptospirosis is a mild, influenza-like illness that may develop into Weil’s syndrome characterized by jaundice, renal failure, haemorrhage and myocarditis. Meningitis and pulmonary haemorrhage with respiratory failure may be seen as well. Laboratory testing is essential because signs and symptoms may resemble those of other common infectious diseases. Humans react to the disease by producing anti-Leptospira antibodies. Seroconversion may occur as early as 5-7 days after the onset of disease. Acute disease is characterized by the presence of specific IgM antibodies. The microscopic agglutination test is the reference test for leptospirosis and detection of specific IgM antibodies may be used for the early diagnosis. The Test-it™ Leptospira IgM lateral flow assay is a relatively simple and rapid assay that may be used as a point-of-care assay. The assay does not require special training, equipment, electricity or refrigeration. Results are obtained in 15 minutes. The assay devices and the running fluid may be stored at +4°C to +28°C.

Principle
The Test-it™ Leptospira IgM lateral flow assay is a one step immunochromatographic lateral flow assay. A lipopolysaccharide antigen (LPS) prepared from a culture of Leptospira is immobilised in a discrete line on a porous nitrocellulose membrane located in the test zone (T). The assay utilises a dried detection reagent deposited within the device. The mobile detection reagent consists of anti-human IgM antibodies labelled with red colloidal gold particles. To perform the assay a serum or whole blood sample is placed in the sample well (S). Running fluid is added to solubilise the detection reagent and to carry the molecules from the sample and detection reagent through the porous membrane in the test zone (T). Antibodies in the clinical specimen that are specific for the pathogen attach to the LPS antigen and these antibodies will be stained by binding of the detection reagent. The presence of specific antibodies will be revealed by the appearance of a red line in the test zone (T) of the assay device. If the sample does not contain pathogen specific IgM antibodies, the sample and detection reagent will pass over the test zone and no line will appear in the test zone. With any sample a red line should always appear in the control zone (C). The control ensures that the detection reagent is still active.

Test Kit and Labelling
Each kit contains 25 individually wrapped assay devices together with 1 bottle of running fluid, sufficient for the analysis of 25 serum or whole blood samples, 25 alcohol swabs, 25 lancets, and 25 plastic pipettes.

Utensils Not Provided: Timer

Storage
Test-it™ Leptospira lateral flow kits should be stored at +4°C to +28°C, in a dry place and protected from direct exposure to sunlight for optimal performance. Individual devices may be stored up to +45°C for short periods.

Expiry Date
The expiry date is printed on the packaging. When stored properly the shelf life is 2 years from date of manufacturing.

Precautions
Blood and serum samples as well as equipment and supplies for specimen handling should be handled with care as they are potentially infectious. Used devices, disposables and samples should be properly decontaminated and discarded.

Specimen collection
Serum should be prepared in the same way as routinely performed for any serological assay. Freshly collected samples should be used. Serum samples stored at -20°C may be used as well. Venipuncture blood may be used if the vacutainer contains an anticoagulant.
Standard Assay Procedure
1. Remove a Test-it™ Leptospira lateral flow assay device from the packaging and place on a bench top with the test window facing upwards.
2. Immediately check if desiccant is still orange in colour. If desiccant has turned green, the test has been exposed to moisture and the test must be discarded.
3. Using the plastic pipette provided, draw up serum or whole blood to the first marked line (5µl) of the plastic pipette and transfer to the round sample port (S) on the cassette device.
4. Immediately add 3 drops of running fluid to the round sample port (S). *NB Pierce the tip of the buffer bottle by screwing the cap down fully
5. You will see the reagent moving across the test and control zones. This shows that the test is working.
6. Read results at 15 minutes. Results are stable for a further 10 minutes; thereafter false results may occur.

Interpretation of Test Results
Positive Result: Indicated by the presence of a line at the test zone (T) and a line at the control zone (C). A positive result is consistent with acute leptospirosis. The sensitivity of the assay and the strength of the staining of the test line depend on the stage of the disease as well as on other factors. As specific antibodies reach detectable levels about one week after the onset of illness, a sample collected very early in the disease may fail to give a positive result in this assay. A staining intensity rated strong provides good evidence of current leptospirosis. If staining is absent or weak and suspicion of leptospirosis remains, the assay should be performed on a second sample taken at a later date in conjunction with a re-examination of the first sample to look for an increase in the amount of antibodies or seroconversion. In endemic areas faint staining at the test zone corresponding to low or borderline titres in ELISA, due to antibodies being present as a result of previous exposure, may be observed. False results may be obtained for rheumatoid factor positive samples.

Negative Result: Indicated by absence of a line at the test zone (T) and presence of a line at the control zone (C). If a negative result is obtained for a sample collected very early in the disease testing of a second sample collected a few days later may show seroconversion.

SPECIAL NOTE:
The intensity of the Control line (C) may vary from test to test. A weak Control line (C) does not disqualify the test and has no influence on the validity of the test result (T) whatsoever.

Limitations of Use
The sensitivity and specificity of the Lepto IgM flow assay depend on various factors including stage of disease and previous antibiotic usage. It is advised that the microscopic agglutination test be used as a confirmatory test.

Further Reading of Suggested Literature