Leptospirosis is a zoonotic disease of worldwide prevalence. Although wild mammals serve as a primary natural reservoir of pathogenic strains, domestic animals serve as a major source of human infection. Animals most often implicated in human disease transmission include dogs, cattle, swine, sheep, goats, horses, and wild rodents. Most often transmission is indirect, by human contact with soil, food, or water contaminated by urine from an animal with leptospirosis.

**SUMMARY AND EXPLANATION OF TEST**

Leptospirosis, infection with pathogenic serogroups of the genus *Leptospira*, is accompanied by a brisk antibody response. Both IgG and IgM class antibodies are detectable with a wide variety of serologic methods. Although over 150 serovars of parasitic leptospires have been classified and organized into 16 serogroups, certain antigens have been found to be broadly cross-reactive throughout all serovars tested. In patients with leptospirosis, antibody is detectable by Leptospirosis IHA within 1 to 2 weeks post-onset of symptoms, peaks at 2 to 4 weeks, and falls below a detectable level within 9 months to a year.

The Focus Diagnostics IHA (indirect hemagglutination) test utilizes an ethanol-extracted antigen first described by Cox in 1957, which has been widely used both in hemagglutination and hemolysis procedures for the diagnosis of human leptospirosis.

**TEST PRINCIPLE**

This indirect hemagglutination test uses human type “O” erythrocytes that have been coated (sensitized) with genus-specific leptospiral antigens derived from *Leptospira biflexa* strain Patoc 1. When a positive patient serum is incubated with these coated cells, the antigen-antibody reaction causes agglutination of the cells. The antibody titer is measured by testing serial dilutions of positive sera and recording the highest serum dilution that produces the agglutination reaction. Control cells (unsensitized erythrocytes) are also tested to screen for non-specific reactivity.

**MATERIALS SUPPLIED**

Focus Diagnostics’ Leptospirosis Antibody IHA test kit contains sufficient materials to perform 120 determinations.

**INTENDED USE**

The Focus Diagnostics Leptospirosis IHA test is intended for the detection and semi-quantitation of human antibodies to the bacteria of the genus *Leptospira*. The kit and its reagents are intended for use as a laboratory aid in the diagnosis of human leptospirosis.
PBS

One vial of Phosphate buffered saline (PBS) powder. Reconstitute with 1 Liter distilled (or purified) water. The reconstituted solution is a 0.01 M buffer at pH 7.2 ± 0.1. Before and after reconstitution, store PBS at 2 to 8°C. Allow to warm to room temperature before use. Do not use if cloudiness, discoloration, or other indications of bacterial contamination are present.

MATERIALS REQUIRED, BUT NOT SUPPLIED

1. Microtiter plates, U-bottom, polystyrene.
2. Test tubes and rack, microcentrifuge tubes or microtiter plate for serum dilutions.
3. Clinical centrifuge
4. 2 to 8°C refrigerator
5. Calibrated pipets or piston-type pipettors with disposable tips capable of accurately delivering 10 to 500 µL.
6. Microtiter droppers, 25 µL and 50 µL.
7. Microdiluters, 50 µL
8. 1 L Volumetric flask or graduated cylinder.
9. Timer
10. Viewing mirror (optional)
11. Plate shaker (optional)

SHELF LIFE AND HANDLING

1. Kits are stable through the end of the month indicated in the expiration date when stored at 2 to 8°C in an upright position.
2. Do not use test kit or reagents beyond their expiration dates.
3. Store the Test Cells and the Control Cells in an upright position.
4. Do not freeze or heat the Test and Control cells.

WARNINGS AND PRECAUTIONS:

1. This kit is for in vitro diagnostic use only.
2. All blood products should be treated as potentially infectious. Source materials from which this product (including negative control) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and decontaminated or disposed of using proper biohazard precautions.
3. Do not substitute or mix reagents from different kit lots or from other manufacturers.
4. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
5. Cross-contamination of patient specimens on a microtiter plate can cause erroneous results. Add patient specimens and handle plates carefully to avoid mixing of sera from adjoining wells.
6. Use aseptic techniques to avoid microbial contamination.

SPECIMEN COLLECTION AND PREPARATION

Serum is the preferred specimen source; however, plasma may also be used. No attempt has been made to assess the assay's compatibility with other specimens. Hyperlipemic, hemolyzed, heat inactivated, or contaminated sera may cause erroneous results; therefore, their use should be avoided.

Specimen Collection and Handling

Collect blood samples aseptically using approved venipuncture techniques by qualified personnel. Allow blood samples to clot at room temperature prior to centrifugation. Aseptically transfer serum to a tightly closing sterile container for storage at 2 to 8°C. If testing is to be delayed longer than 5 days, the sample should be frozen at –20°C or colder. Freeze-thaw damage can result if specimens are frozen in self-defrosting freezers. Thaw and mix samples well prior to use.

Specimen Preparation

Prepare 1:50 screening dilutions of test serum specimens as follows:

Mix 10 µL of patient serum to 490 µL PBS. Mix by gentle swirling or repeated pipetting of the serum dilution.

TEST PROCEDURE

Screen patient specimens at 1:50. Specimens positive at this dilution should be titrated.

Screening Test Procedure

1. Prepare components for use. Label the microtiter plate, allowing two wells for each patient or control sera. The first well of each pair will be a control well, the second a test well. Include sets of wells for Positive and Negative Controls.
2. Add 50 µL of Positive Control, as bottled, to both a Control Well and a Test Well.
3. Add 50 µL of Negative Control, as bottled, to both a Control Well and a Test Well.
4. Add 50 µL of the diluted (1:50 screening dilution) sample (see Specimen Preparation, above) of each specimen to both the control and test wells.
5. To all control wells, add 25 µL Control Cells. Mix cells well by swirling before adding.
6. To all test wells, add 25 µL Test Cells. Mix cells well by swirling before adding.
7. Mix plate immediately, either by tapping the plate edges for 60 seconds or by mechanically shaking the plate for 15 to 30 seconds.
8. Cover plate with clear plastic tape or with another microtiter plate to prevent evaporation.
9. Incubate plate at room temperature (20 to 25°C) on a vibration-free level surface for 1 hour.
10. Read agglutination patterns. Reading may be delayed for 2 hours if plate is left undisturbed.

Titration Procedure

1. Label each microtiter plate, allowing a full 12-well row for each specimen.
2. Add 50 µL Titration Buffer to wells #3 through 12 of each horizontal row.

3. For each serum specimen, add 50 µL of the diluted (1:50 screening dilution) sample (see Specimen Preparation, above) to wells #1 to 3. Include Positive and Negative Controls, diluted through their respective reference titers.

4. With 50 µL microtiter diluting loops (or with mechanical pipettor), serially dilute each serum, starting with well #3. This procedure consists of mixing well #3 and transferring 50 µL from well #3 to well #4, then repeating this mix and transfer step for wells #4 to 12. The 50 µL removed from well #12 is discarded.

5. Add 25 µL Control Cells to the first well of each row. Mix well by swirling before adding.

6. Add 25 µL Test Cells to all other wells (#2 to 12). Mix well by swirling before adding.

7. Mix plate immediately, either by tapping the plate edges for 60 seconds or by mechanically shaking the plate for 15 to 30 seconds.

8. Cover plate with clear plastic tape or with another microtiter plate to prevent evaporation.

9. Incubate plate at room temperature (20 to 25°C) on a vibration-free level surface for 1 hour.

10. Read agglutination patterns. Reading may be delayed for 2 hours if plate is left undisturbed.

QUALITY CONTROL

Each assay run should include both Positive and Negative controls, and the controls should be run in Test Wells and Control Wells. When performing patient specimen titration, titer the Positive Control through the reference range in the Test Wells.

1. The Positive Control should be negative on the Control Well.

2. The Positive Control should demonstrate ≥ 2+ agglutination pattern on the screening Test Well.

3. When titration is performed, the Positive Control should endpoint (1+ Agglutination pattern) 4 to 16-fold beyond the bottled dilution.

4. The Negative Control should be negative on both the Control and the Test Wells.

If controls do not exhibit these results, sample results should be considered invalid and the assay repeated.

INTERPRETATION OF TEST RESULTS

Read control wells first during every run to ensure correct interpretation.

Reading Agglutination Patterns

Positive wells are defined as wells showing ≥ 1+ agglutination. Read the Agglutination Pattern on each well, and grade the agglutination as follows:

Positive Reactions

4+ An extremely granular reaction covering bottom of well.
3+ A solid mat on the bottom of the well, with folded edges.
2+ A solid mat with irregular edges.
1+ A granular or irregular ring covering more than 50% of well bottom and distinctly different than control well reaction: equivalent to that observed for the Positive Control at its reference endpoint titer.

Negative Reaction

0 Smooth compact button to small ring similar to a doughnut: equal to that observed in the Negative Control well.

Interpreting the Patient Specimen Results

Control Wells: The control well should be negative. If positive, the specimen is showing non-specific reactivity and should be treated to remove this activity (see Non-specific Reactions, below) or reported out as non-specific.

Test Wells:

<1:50 Negative No serologic evidence of leptospirosis.

1:50 Borderline positive. Patients should be evaluated for clinical correlation with active or recent leptospirosis. Follow-up specimens should be ordered for serology and isolation of live leptospires.

≥1:100 Positive for leptospiral antibody Serological evidence of active or recent leptospirosis.

Non-specific Reactions

If the patient control wells shows 1+ or greater agglutination, the serum sample may be further treated to remove this reactivity (absorption with control cells “O”), or tested by an alternate method. An absorption method utilizing Control Cells is as follows:

1. Add 20 µL serum to 80 µL Control Cells in a 12 x 75 mm test tube.

2. Incubate tube at 56°C for 15 minutes, then centrifuge to pellet cells.

3. Transfer 20 µL supernatant to another 12 x 75 mm test tube containing 180 µL Control Cells and incubate another 15 minutes at 2 to 8°C.

4. Centrifuge tube to pellet cells, and remove absorbed serum for repeat testing.

LIMITATIONS

1. Sera showing non-specific reactions (control cell agglutination) that cannot be eliminated by suggested protocol cannot be accurately tested by this procedure. They should be tested using an alternate method or reported out as non-specific.

2. All results from this test must be correlated with clinical data.

3. Sera drawn too close to onset of symptoms may precede the initial IgM antibody response.

4. Acute titers may be delayed or substantially decreased by early and intensive antibiotic treatment.

5. This procedure detects genus-specific antibody and cannot be used for determination of the infecting serovar. Determination of the infecting serovar can only be definitively established by isolation and typing of the live leptospire.
This test has been standardized for testing human sera only. Samples from animals may not give accurate results.

EXPECTED VALUES

Serum specimens from healthy persons or those with a diagnosis other than leptospirosis should have titers < 1:50. In patients with leptospirosis, acute phase antibody (IgM class) is detectable by Leptospirosis IHA within 1 to 2 weeks post-onset of symptoms and peaks at 2 to 4 weeks. Titers are generally >1:200 before falling. Titers generally fall below detectable levels within 9 months to a year.

SPECIFIC PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

A panel of human sera were tested with both the Focus Diagnostics Leptospirosis IHA Test and reference Leptospira IHA reagents\(^1\) prepared at the Centers for Disease Control, Atlanta, Georgia. Of the 103 specimens tested, 82 were negative by both procedures and 19 were positive by both procedures. The two discrepant specimens had a titer of 1:50 in the CDC test and 1:100 in the Focus Diagnostics test. The overall sensitivity compared to the CDC reagents was 100% and the specificity was 97%. Of 19 sera positive by both tests 6 had the same titer, 11 were within 1 dilution and 2 were within 2 dilutions. In 12 of 13 specimens where there was a titer difference, the Focus Diagnostics titer was higher.

Method Comparison

In a published comparison\(^6\), the IHA test and the slide agglutination test were compared to the reference microscopic agglutination test. The sensitivity of the IHA procedure was 92%, as compared with 69% for the slide agglutination test. The specificity for the IHA was 95%, in contrast to 83% for the slide test. The IHA test detected antibodies in 11 different serogroups of *Leptospira*. This indicates that the IHA test is a sensitive and specific test for detecting group-specific antibodies to *Leptospira*.

REFERENCES