See external Label

AccuDiag™
Leptospira IgG
ELISA Kit

<table>
<thead>
<tr>
<th>Test</th>
<th>Leptospira IgG ELISA</th>
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</thead>
<tbody>
<tr>
<td>Method</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>Principle</td>
<td>Indirect; Antigen Coated Plate</td>
</tr>
<tr>
<td>Detection Range</td>
<td>Qualitative : Positive, Negative Control</td>
</tr>
<tr>
<td>Sample</td>
<td>10 µL</td>
</tr>
<tr>
<td>Total Time</td>
<td>~ 15 min.</td>
</tr>
<tr>
<td>Shelf Life</td>
<td>12 Months from the manufacturing date</td>
</tr>
<tr>
<td>Specificity</td>
<td>87.5%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>80%</td>
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</tbody>
</table>

**INTENDED USE**

The Diagnostic Automation, Inc. Leptospira ELISA test is a qualitative enzyme immunoassay for the detection of antibodies to Leptospira in samples of human serum or plasma. This test is intended to be performed by trained medical technologists only.

**SUMMARY AND EXPLANATION**

The clinical manifestations of leptospirosis range from a mild catarrh-like illness to icteric disease with severe liver and kidney involvement. Natural reservoirs for leptospirosis include rodents as well as a large variety of domesticated mammals. The organisms occupy the lumen of nephritic tubules in their natural host and are shed into the urine. Human infection derives from direct exposure to infected animals (veterinarians, abattoir workers, or dairy workers for example) or by exposure to environments contaminated by animal carriers (e.g. agricultural workers). Bathing or swimming in water sources about which livestock have been found to be 10 to 12 days. Antibody levels then gradually recede but may remain detectable for years.

Epidemiologic factors, clinical findings, exposure in endemic regions and other laboratory results should be considered in diagnosing acute disease. Acute disease diagnosis will also include a positive laboratory confirmation in many cases. This test is designed to measure acute infections with Leptospira. Confirmation of a positive sample by additional methods should be followed.

**TEST PRINCIPLE**

The microwells are coated with purified Leptospira biflexa antigen. During the first incubation with the diluted patients’ sera, any antibodies which are reactive with the antigen will bind to the coated wells. After washing to remove the rest of the sample, the Enzyme Conjugate is added. If antibodies have been bound to the wells, the Enzyme Conjugate will then bind to these antibodies. After another series of washes, a chromogen (tetramethylbenzidine or TMB) is added. If the Enzyme Conjugate is present, the peroxidase will catalyze a reaction that consumes the peroxide and turns the chromogen from clear to blue. Addition of the Stop Solution ends the reaction and turns the blue color to a bright yellow color. The reaction may then be read visually or with an ELISA reader.

**SPECIMEN COLLECTION AND PREPARATION**

Serum or plasma may be stored at 2-8 °C for up to five days. Serum may be frozen below -20 °C for extended periods. Freezing whole blood samples is not advised. Do not heat inactivate samples and avoid repeated freezing and thawing of samples.

**MATERIALS AND COMPONENTS**

Materials provided with the test kits

1. Plate: Microwells containing Leptospira antigens - 96 test wells in a test strip holder.
2. Enzyme Conjugate: One (1) bottle containing 11 ml of anti-human IgG (gamma chain antibody) conjugated to peroxidase.
3. Positive Control: One (1) vial containing 1 ml of diluted positive human serum.
4. Negative Control: One (1) vial containing 1 ml of dilution buffer.
5. Chromogen: One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
6. Wash Concentrate 20X: One (1) bottle containing 25 ml of concentrated buffer and surfactant.
7. Dilution Buffer: Two (2) bottles containing 30 ml of buffered protein solution.
8. Stop Solution: One (1) bottle containing 11 ml of 1 M phosphoric acid.

Materials required but not provided

1. Micropipette
2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Reagent grade (DI) water
4. Graduated Cylinder
5. Sample Dilution Tubes
6. Absorbent paper
7. ELISA plate reader with a 450 nm and a 620-650 nm filter (optional if results are read visually).

**Proper Temperature**

All incubations are at room temperature (15-25°C).

**Preparation**

- Before use, bring all reagents and samples to room temperature (15-25 °C) and mix.
- (20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature and mixed. **Ensure that (20X) Wash Concentrate is completely in solution before diluting to working concentration.** To dilute (20X) wash concentrate to working dilution, remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.
QUALITY CONTROL

The use of controls allows validation of kit stability. The kit should not be used if any of the controls are out of range.

Expected values for the controls are:

<table>
<thead>
<tr>
<th></th>
<th>Reference Method *</th>
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</thead>
<tbody>
<tr>
<td>Negative</td>
<td>-</td>
</tr>
<tr>
<td>Positive</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Automation,Inc.</td>
<td></td>
<td>28</td>
</tr>
</tbody>
</table>

Positive Agreement: 80% (8/10)
Negative Agreement: 87.5% (28/32)

*Reference Method refers to a commercially available ELISA.

LIMITATIONS OF PROCEDURE

Diagnosis of Leptospira infection should not be made solely based on results of the ELISA test alone, but in conjunction with other clinical signs and symptoms and other laboratory findings.

Epidemiologic factors, clinical findings, exposure to endemic regions, and other laboratory results should be considered when making a diagnosis.

EXPECTED VALUES

The number of antibody positive subjects in a population depends on two factors: disease prevalence and clinical criteria used to select the tested population. Because very few positives should be seen in a randomly screened population in a non-endemic area, most serology tests are not specific enough to screen non-endemic populations. Even in an endemic region, serology screening often yields many false positives if used to randomly screen patients. Serology tests are useful to test patients in an endemic region with signs and symptoms consistent with the disease.

Antibody levels are generally low or absent during very early infection. Symptomatic patients may have no antibody during the first 1-2 weeks after exposure and the antibody titer will rise with time.

PRECAUTIONS

1. Do not deviate from the specified procedures when performing this assay. All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
2. For In Vitro Diagnostic Use Only.
3. Do not interchange reagents between kits with different lot numbers.
4. Do not use reagents that are beyond their expiration dates. Expiration dates are specified procedures may affect the sensitivity and specificity of the assay.
5. Unused microwells should be stored in the desiccated pouch to protect them from moisture.
6. Do not use solutions if they precipitate or become cloudy. Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
7. Do not add azides to the samples or any of the reagents.
8. Controls and some reagents contain Thimerosal as a preservative, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.

9. Do not use serum that may have supported microbial growth, or is cloudy due to high lipid content. Samples high in lipids should be clarified before use.

10. Treat all reagents and samples as potentially infectious materials. Positive control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. Use care to prevent aerosols and decontaminate any spills of samples.

11. Stop solution is a 5% solution of phosphoric acid in water. If spilled on the skin, wash with copious amounts of water. If acid gets into the eyes, wash with copious amounts of water and seek medical attention.

**Storage Conditions**

1. Reagents, strips and bottled components should be stored at 2-8 °C.

2. Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25 °C).

**REFERENCES**


