Leptospira IgM ELISA Kit

Catalog Number KA3297
96 assays
Version: 03

Intended for research use only
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Introduction

Intended Use

The *Leptospira* IgM ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of antibodies to *Leptospira* biflexa (serovar patoc 1) in serum and plasma. The *Leptospira* IgM ELISA test is for use only by a laboratory.

Background

Leptospira infections in humans are transmitted from infected animals, or by exposure to areas inhabited by contaminated animals. Another source of infection is derived from swimming or bathing in contaminated water, as the infection enters through mucosal eye surfaces or skin contusions. Infected animals may include rats and mice, but also larger mammals such as dogs, sheep, cows, deer, rabbits, etc. are primary sources of infection. The leptospira bacteria is shed through the urine of infected animals. Incubation period of the infection in humans is normally 10 to 12 days, but can also range from 3 to 30 days. Antibody detection occurs around the 6th to 10th day of disease, and within 3 to 4 weeks, they reach their peak level. Even though antibody levels slowly abate, they may persist and show up in diagnosis years afterward.

The extent of symptoms can range anywhere from mild inflammation of the mucous membrane to jaundice, where severe kidney and liver infections are detected. When diagnosing Leptospira infections, it is important to review other factors, such as clinical findings, epidemiology, and the particular region in which the infection occurred. These methods should be considered in acute cases. The Leptospira ELISA kit is the ideal test for acute leptospirosis infections.

Principle of the Assay

The principle of the *Leptospira* IgM ELISA test is a three-incubation process whereby the first incubation involves the coating of the wells with purified Leptospira Patoc 1 antigen. During this step, any antibodies that are reactive with the antigen, will bind to the coated wells. Next, the wells must be washed to remove test sample. At this point Enzyme Conjugate is added. During this second incubation, the Enzyme Conjugate will bind to any antibodies present. Before the third incubation step, more washings are necessary. Then a chromogen (tetramethylbenzidine or TMB) is added. With the presence of Enzyme Conjugate and the peroxidase causing the consumption of peroxide, the chromogen changes to a blue color. The blue color turns to a bright yellow color after the addition of the stop solution, which ends the reaction. ELISA readers can be used to obtain results, or results may be read visually.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Strips: Microwells containing <em>Leptospira</em> antigen - 96 test wells in a test strip holder.</td>
<td>96 wells</td>
</tr>
<tr>
<td>Enzyme Conjugate: One bottle containing anti-human IgM antibody conjugated to peroxidase.</td>
<td>11 ml</td>
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<tr>
<td>Positive Control: One vial containing diluted positive human serum.</td>
<td>1 ml</td>
</tr>
<tr>
<td>Negative Control: One vial containing dilution buffer.</td>
<td>1 ml</td>
</tr>
<tr>
<td>TMB Substrate Solution: One bottle containing the chromogen tetramethylbenzidine (TMB).</td>
<td>11 ml</td>
</tr>
<tr>
<td>RF Absorbent: One bottle containing goat anti-human IgG.</td>
<td>5 ml</td>
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<tr>
<td>Wash Concentrate (20X): One bottle containing concentrated buffer and surfactant.</td>
<td>25 ml</td>
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<tr>
<td>Dilution Buffer: Two bottles containing buffered protein solution.</td>
<td>30 ml x 2</td>
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<tr>
<td>Stop Solution: One bottle containing 0.73 M phosphoric acid.</td>
<td>11 ml</td>
</tr>
</tbody>
</table>

Storage Instruction

✔ Reagents, strips and bottled components: Store between 2-8°C.
✔ Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25°C).

Materials Required but Not Supplied

✔ Pipettes
✔ Squeeze bottle for washing strips (narrow tip is recommended)
✔ Reagent grade water and graduated cylinder
✔ Tubes for Sample dilution
✔ Absorbent paper
✔ ELISA plate reader with a 450 nm and a 620-650 nm filter (optional if results are read visually.)

Precautions for Use

✔ 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
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Do not interchange reagents between kits with different lot numbers.

Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.

Unused microwells should be stored in the desiccated pouch to protect them from moisture.

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Do not add azides to the samples or any of the reagents.

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.

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.

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

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.

Persons who are color blind or visually impaired may not be able to read the test visually and should use Spectrophotometric readings to interpret results.
Assay Protocol

Sample Preparation

The *Leptospira* IgM ELISA test should be performed on serum. Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation from the clot. Serum may be stored at 2-8 ºC for up to five days. Serum may be frozen below -20 ºC for 3-6 months. Freezing whole blood samples is not advised. Do not heat inactivate samples and avoid repeated freezing and thawing of samples. Limpemic and strongly hemolytic serum should be avoided.

Single specimens are used to assess exposure; two specimens collected at different times from the same individual are used to show sero-conversion. Paired specimens should be tested at the same time. It is recommended that a convalescent specimen be collected from patients showing either an initially non-reactive result or a weakly reactive result.

Assay Procedure

✓ Ensure all samples and reagents are at room temperature (15-25°C)
✓ When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each step should help to minimize bubbles in the wells.
✓ Negative and positive controls are supplied pre-diluted. DO NOT dilute further.
✓ DO NOT add RF Absorbent to negative and positive controls.

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Dilute sera 1:40 using the Dilution Buffer (e.g. 10 μl sera and 390 μl dilution buffer).
3. Gather the required number of sample dilutions tubes. Add 100 μL of diluted samples to the appropriate tubes. Add 40 μL RF Absorbent to each tube. Mix well.
4. Incubate at room temperature for 5 minutes. Add 100 μL negative control to well # 1, 100 μL positive control to well # 2 then transfer all 140 μL of each sample tube to the remaining wells.
5. Incubate at room temperature for 10 minutes, then wash. *After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer.
6. Add 100 μl of Enzyme Conjugate to each well.
7. Incubate at room temperature for 10 minutes, then wash.*After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer.
8. 100 μL of the Chromogen to each well.
9. Incubate at room temperature for 5 minutes.
10. 100 μL of the Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger for approximately 15 seconds.
11. Read within one hour of adding Stop Solution.

*Washings consist of vigorously filing each well to overflowing and decanting contents three (3) separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.
Data Analysis

Calculation of Results

✓ Reading Results
• Visually: Look at each well against a white background (e.g. paper towel) and record as clear or +, ++ or +++ reaction.
• ELISA Reader: Zero reader on air. Set for bichromatic readings at 450/620-650 nm.

✓ 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:
• Negative - 0.0 to 0.3 OD units
• Positive - 0.5 OD units and above

✓ Interpretation of the Test
• Initially Non-reactive: Samples interpreted as non-reactive (0.0-0.3 OD units, or zero color) indicate antibody is not present in the sample. Since antibody may not be present during early disease, (5-8 days incubation), confirmation 2-3 weeks later is indicated for laboratory diagnosis. At this later time, individuals showing weak reactons (0.3-1.0 OD or +,++) should be further tested by alternate methods or retested 10-14 days later. A convalescent serum with a significant reaction (>1.0 OD) indicates the formation of specific antibody against leptospira. An initially negative result followed by a positive result implies seroconersion.
• Initially Weakly Reactive: Weakly reactive specimens should be cautiously interpreted. In normal populations, weakly reactive samples are infrequent but possible. Confirmation using a sample collected 2-3 weeks later (paired acute and convalescent sera) is recommended. >1.0 OD in the second sample confirms the presence of recent, specific antibody. [Caution: If this is a cross-reactive antibody, the convalescent serum sample may not show a higher antibody level than the acute sample.] If sample reading remains at 0.3-1.0 OD, or +,++, a second methodology should be considered, or the sample may be interpreted as taken beyond rising titer (titer declining).
• Initially Reactive: Samples interpreted as strongly reactive (>1.0 OD or ++++) may indicate the presence of specific antibody.
Performance Characteristics

A total 79 serum samples were tested against another commercial ELISA. The following results were obtained.

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<tr>
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<th>ELISA +</th>
<th>ELISA -</th>
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<tr>
<td>Abnova ELISA +</td>
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<td>0</td>
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<tr>
<td>Abnova ELISA -</td>
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<td>65</td>
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- Positive Agreement: 100% (14/14)
- Negative Agreement: 100% (65/65)
Resources

Troubleshooting

Negative control has excessive color after development.

✓ Reason: inadequate washings
✓ Correction: wash more vigorously. Remove excessive liquid from the wells by tapping against an Absorbent towel.
✓ Do not allow test wells to dry out.

References

Plate Layout

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