Leptospira IgM ELISA

Lepto-M-96

Intended Use

The LEPTOSPIRA Microwell ELISA test is an enzyme immunoassay for the detection of antibodies to the *Leptospira* genus for the serological confirmation of infections in serum and plasma. This test is intended to be performed by trained laboratory personnel 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 pastured has been demonstrated to be a potential infection hazard. The organisms enter the host through skin abrasions, mucosal surfaces or the eye. The incubation period can range from 3 to 30 days but is usually found to be 10 to 12 days. Antibodies can become detectable by the 6th to 10th day of disease and generally reach peak levels within 3 to 4 weeks. 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 *Leptospir*a. Confirmation of a positive sample by additional methods should be followed.

Assay 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.

Reagents

Item	Description	Symbol
Test Strips	Microwells containing Leptospira antigens - 96 test wells in a test strip holder.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11 ml of anti-human IgM (μ chain antibody) conjugated to peroxidase.	CONJ
Positive Control	One (1) vial containing 1 ml of diluted surrogate positive.	CONTROL +
Negative Control	One (1) vial containing 1 ml of diluted negative human sera.	CONTROL −
Chromogen	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).	SUBS TMB
RF Absorbent	One (1) bottle containing 5 ml of goat anti-human IgG.	Ab
Wash Concentrate (20X)	One (1) bottle containing 25 ml of concentrated buffer and surfactant.	WASH BUF
Dilution Buffer	Two (2) bottles containing 30 ml of buffered protein solution.	SPECM DIL
Stop Solution	One (1) bottle containing 11 ml of 1 M phosphoric acid.	SOLN

Statement Of Warnings

- **For Export Only**
- **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.
- For In Vitro Diagnostic Use Only.
- 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.
- Do not use solutions if they precipitate or become cloudy.
  Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
- 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. Controls have 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.

Storage

- Reagents, strips and bottled components should be stored at 2-8 °C
- Squeeze bottle containing diluted wash buffer may be stored 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.

Specimen Collection And Handling

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

Procedure

Material Provided

Leptospira IgM ELISA Kit

Materials Required But Not Provided

- Micropipette
- Squeeze bottle for washing strips (narrow tip is recommended)
- Reagent grade (DI) water
- Graduated cylinder
- Sample Dilution Tubes
- Absorbent paper

Suggested Materials

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)

Test Procedure

Notes:

- 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.

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Dilute patient sera 1:40 using the Dilution Buffer (e.g. 10 µl sera and 390 µl dilution buffer). Treat each patient's sera with RF Absorbent by adding 100 ul of the diluted sample to 40 ul of the RF Absorbent in a small tube, DO NOT PERFORM THIS STEP IN THE TEST MICROWELLS. Mix well and incubate at room temperature for 5-10 minutes. Then move on to step #3. Kit controls do not need to be RF treated.
3. Add 100 ul of Negative Control to well #1 and 100 ul of Positive Control to well #2. Then add **100 µl** of diluted (and RF treated) samples to the corresponding wells.
4. Incubate in the test wells for **10 minutes**, then wash.* After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer.
5. Add **100 µl** of Enzyme Conjugate to each well.
6. 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.
7. Add **100 µl** of the Chromogen to each well. Incubate at room temperature for **5 minutes**.
8. Add **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**.
9. Read within one hour of adding Stop Solution.

* Washings consist of vigorously filling 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.

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 0.20 OD units
Positive - 0.50 OD units and above

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.

Interpretation Of The Test

Zero ELISA reader on air. Read all wells at 450/650 to 620 nm.

A reactive OD reading indicates that the patient may be infected by *Leptospira* or a closely related organism.
A non-reactive OD reading indicates that the patient has no detectable level of antibodies. This may be due to lack of infection or poor immune response by the patient.

Interpretation of Results -Visual

Compare results to the controls.
A sample should be interpreted as non-reactive if there is little to no color development.
A sample should be considered weakly reactive (+ to ++) if there is obvious color development but not as strong as the positive control.
A sample should be considered reactive if the color development (+++) is near or greater than the positive control.

Initially Non-reactive: Samples interpreted as non-reactive (0.0-0.15 OD units, or little or zero color) indicate antibody is not present in the sample. Since antibody may not be present during early disease confirmation 2-3 weeks later is indicated for laboratory diagnosis.

Initially Weakly Reactive: Weakly reactive specimens should be cautiously interpreted. In normal populations, weakly reactive (0.15 to 1.00 OD) samples are infrequent but possible.

Initially Reactive: Samples interpreted as strongly reactive (>1.0 OD or +++) may indicate the presence of specific antibody. Antibody presence alone cannot be used for diagnosis of acute infection, since antibodies from prior exposure may circulate for a prolonged period of time.

Limitations Of The 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.

Performance Characteristics

		Reference Method*	
		+	-
Eiger	+	22	0
	-	0	81

Positive Agreement: 100% (22/22)
Negative Agreement: 100% (81/81)

*Reference Method refers to a commercially available ELISA.

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