Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ Leptospira IgG& IgM ELISA Kit

REF 8210-35

IVD See external Label 2°C 8°C 96 Tests

Test	Leptospira IgG& IgM ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Qualitative : Positive, Negative Control
Sample	10 µL
Total Time	~ 20 min.
Shelf Life	12 Months from the manufacturing date
Specificity	87.5 %
Sensitivity	88.7%

INTENDED USE

The Leptospira IgG & IgM Microwell ELISA test is an enzyme immunoassay for the detection of antibodies to *Leptospira biflexa* (serovar *patoc 1*) 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 leptospira. Confirmation of a positive sample by additional methods should be followed.

TEST PRINCIPLE

The microwells are coated with purified Leptospira Patoc 1 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

The Leptospira Microwell ELISA test should be performed on serum or plasma. Serum may be stored at 2-8 ºC for up to five days. Serum may be frozen below -20 ºC for extended periods. Do not heat inactivate samples and avoid repeated freezing and thawing of samples.

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.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Plate:** Microwells containing Leptospira antigen - 96 test wells in a test strip holder.
2. **Enzyme Conjugate**: One (1) bottle containing 11 ml of anti-human IgG/IgM antibody conjugated to peroxidase.
3. **Positive Control**: One (1) vial containing 1 ml of diluted surrogate positive control.
4. **Negative Control:** One (1) vial containing 1 ml of diluted negative human serum.
5. **TMB Substrate Solution:** One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
6. **Wash Concentrate:** 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. Pipettes
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 (15-25ºC).

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.

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.

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 in Dilution Buffer (e.g. 10 µl sera and 390 µl dilution buffer). Add **100 µl** of negative control to well #1, **100 µl** of positive control to well #2 and **100 µl** of the samples to the remaining wells.
3. 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.
4. Add **2 drops** of Enzyme Conjugate to each well.
5. Incubate at room temperature for **5 minutes**, then wash.* After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer.
6. Add **2 drops** of the Chromogen to each well.
7. Incubate at room temperature for **5 minutes**.
8. Add **2 drops** 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.

RESULTS

Interpretation of Results –Visual 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.

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.

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

PERFORMANCE CHARACTERISTICS

		Reference Method *	
		+	-
Diagnostic Automation,Inc.	+	14	6
	-	2	47

Positive Agreement: 87.5% (14/16)
Negative Agreement: 88.7% (47/53)

*Reference Method refers to a commercially available ELISA.

LIMITATIONS OF PROCEDURE

Serologic results are an aid in diagnosis but cannot be used as the sole method of diagnosis.
The ELISA has been tested against many serovars, but cannot guarantee that all strains will react equally.

Do not use in veterinary samples.

Treatment is often indicated prior to completion of serologic diagnosis, which requires at least two weeks. Acute leptospirosis must be treated immediately and should not wait for serological confirmation. Diagnosis of leptospira infection should not be made 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.

Many strains and serovars of leptospira are known. Many of the strains are geographically dominant in some areas and not in others. Biflexa Patoc 1 is known to cross react with most serovars **but usually does not cross-react with animal strains.** The relative strength of the reactions may vary by antigen. This must be considered during interpretation. Use of culture or the MAT test is recommended for confirmation as these test are serovar specific.

Since serological assay methods may yield different results for weakly reactive samples, a second serological method (i.e. an alternative method that separately identifies IgM and IgG antibody) is recommended.

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 on each reagent label. Use of reagents beyond their expiration dates may affect results.
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. 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.
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.
12. 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.

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).

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