CE

Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ Leptospira IgG ELISA Kit

Cat # 8204-35

Test	Leptospira IgG ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Qualitative : Positive, Negative Control
Sample	10 µL serum/plasma
Total Time	~ 25 min.
Shelf Life	12 Months from the manufacturing date
Specificity	100%
Sensitivity	100%

INTENDED USE

The **Leptospira IgG 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 IgG ELISA test is for use only by a laboratory.

SUMMARY AND EXPLANATION

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 are 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.

TEST PRINCIPLE

The principle of the Leptospira 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 4wi bind to any antibodies present. Before the third incubation step, more washing is 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.

SPECIMEN COLLECTION AND PREPARATION

The DAI 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. **Conjugate AH IgG (Fc) HRP:** One (1) bottle containing 11 ml of anti-human IgG 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 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 (15-25ºC).

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.

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ASSAY 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 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 100 µl of Enzyme Conjugate to each well.
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 the Chromogen to each well.
7. 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.

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.

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

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, patients showing weak reactions (0.3-1.0 OD or +, ++) should be further tested by alternate methods or re-tested 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 seroconversion.

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. Antibody presence alone cannot be used for diagnosis of acute infection, since antibodies from prior exposure may circulate for a prolonged period of time.

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.	+	12	0
	-	0	65

Positive Agreement: 100% (12/12)
Negative Agreement: 100% (65/65)

*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.

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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

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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