AccuDiag™ Leishmania ELISA Kit

Cat # 8203-35

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

Test	Leishmania ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Qualitative Positive ; Negative Control
Sample	10 µL serum
Total Time	~ 25 min.
Shelf Life	12 Months from the manufacturing date
Specificity	84%
Sensitivity	97%

INTENDED USE

The **Leishmania ELISA Kit** is an enzyme-linked immunosorbent assay (ELISA) for the qualitative screening of IgG antibodies to visceral *Leishmania* in serum.

SUMMARY AND EXPLANATION

Leishmania is a widespread disease affecting millions of people around the world. Globally, Leishmania is a serious infection that has spread over several continents, particularly Europe, India, Asia, Africa, and the Middle East. Transmission of Visceral Leishmaniasis, or VL, is induced by the bite of an infected sandfly, a parasitic member of the *L. donovani* complex. The sand fly is the host of Leishmania after it has already been in contact with an infected agent, such as a dog. Because visceral leishmaniasis attacks visceral organs such as the liver and spleen and the immune system, typically Leishmania has been closely associated with AIDS infections. Visceral Leishmania is a serious disease with high mortality rates.

Several testing methods have been employed to diagnose acute Visceral Leishmaniasis. For example, indirect immunofluorescent antibody tests (IFAT) and direct agglutination tests (DAT) are two serodiagnostic procedures in practice because the anti-leishmanial antibody titers are generally high at the acute stages. One method that is less successful is the aspiration of bone marrow. Besides being a painful and risky procedure, its success rate is not very high. Alternatively, the ELISA method is the favored serodiagnostic procedure, which is sometimes used in conjunction with the IFAT and DAT.

TEST PRINCIPLE

The principle of the Leishmania ELISA test is a three-incubation process whereby the first incubation involves the coating of the wells with Leishmania antigen. During this step, any antibodies that are reactive with the antigen, will bind to the 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.

SPECIMEN COLLECTION AND PREPARATION

Coagulate blood and remove serum. Freeze sample at -20 °C or lower if not used immediately.
Do not heat inactivate serum.
Avoid repeated freezing and thawing of samples.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Plate:** Microwells containing Leishmania antigens - 96 test wells in a test strip holder.
2. **Enzyme Conjugate**: One (1) bottle containing 11 ml of anti-human Ig-Peroxidase (HRP) in a stabilizing buffer with Thimerosal.
3. **Positive Control**: One (1) vial containing 1 ml of diluted Leishmania-positive human sera in buffer with Thimerosal.
4. **Negative Control:** One (1) vial containing 1 ml of diluted Leishmania-negative human sera in buffer with Thimerosal.
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. Pipettes
2. Squeeze bottle for washing strips
3. Dl Water
4. ELISA plate reader with a 450 nm and a 620-650 nm filter(optional if results are read visually
5. Tubes for serum dilutions

Preparation

Wash Buffer - Remove cap and add contents of bottle to 475 ml of reagent grade water. Place diluted wash buffer into a squeeze bottle.

Note: Washings consist of filling to the top of each well, shaking out the contents and refilling.
Avoid generating bubbles in the wells during the washing steps.

Test samples: Make a 1:40 dilution of patients' sera using the dilution buffer.

ASSAY PROCEDURE

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Add 100 µl of negative control to well #1, 100 µl of positive control to well #2, and 100 µl of the diluted (1:40) test samples to the remaining wells.
   Note: Negative and positive controls are supplied as prediluted. Do not dilute further.
3. Incubate at room temperature (15 °C to 25 °C) for 10 minutes.
4. Shake out contents and wash 3 times with diluted wash buffer.*
5. Add 2 drops of enzyme conjugate to each well.
6. Incubate at room temperature for 10 minutes.
7. Shake out contents and wash 3 times with wash buffer.
8. Add 2 drops of Chromogen to every well.
9. Incubate at room temperature for 5 minutes.
10. Add 2 drops of stop solution. Mix wells by tapping plate.

11. Zero ELISA reader on air, read wells at 450 nm with a reference filter at 620-650 nm or read results visually.

* Washings consist of using the diluted wash buffer to fill to the top of each well, shaking out the contents and refilling the wells for a total of 3 times.

Avoid generating bubbles in the wells during the washing steps.

Controls must be included each time the kit is run.

RESULTS

Spectrophotometer:
Zero ELISA reader on air. Read all wells using a bichromatic reading with filters at 450 nm and 620-650 nm.
Positive - Absorbance reading greater or equal to 0.2 OD units.
Negative - Absorbance reading less than 0.2 OD units.

Visual
A sample should be interpreted as positive if the degree of color development is obvious and significant.

Troubleshooting

Problem: Negative control has substantial color development.
***Correction*:** Inadequate washings. Rerun test with more vigorous washings.

QUALITY CONTROL

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must be over 0.3 OD units and the negative control must be under 0.2 units. Should the values fall outside these ranges, the kit should not be used.

PERFORMANCE CHARACTERISTICS

		Reference Method *	
		+	-
Diagnostic Automation,Inc.	+	30	10
	-	1	53

Sensitivity: 30/31 = 97%
Specificity: 53/63 = 84%

LIMITATIONS OF PROCEDURE

Serological results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.
Although no specific cross reactions have been recorded to date, reactions by similar organisms cannot be ruled out.

PRECAUTIONS

1. Controls and dilution buffer are casein based buffer and will appear cloudy. In addition, a gelatinous plug may develop at the bottom of the vial. This is normal and does not affect the assay.
2. Wash concentrate may show crystallization upon storage at 4 °C. Crystallization will disappear after diluting to working strength.
3. 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.
4. Do not add azides to the samples or any of the reagents.
5. Controls and some reagents contain Thimerosal as a preservative.
6. Treat all sera as if capable of being infectious.
7. The controls has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. Since no test can offer complete assurance that infectious agents are not present, this product should be used under appropriate safety conditions that would be used for any potentially infectious agent.

STORAGE

Reagents, strips and bottled components:
Store between 2 – 8 ºC.
Squeeze bottle containing diluted wash buffer may be stored at room temperature.

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