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Leishmania IgG ELISA

Leish-G-96

FOR EXPORT ONLY

Intended Use

For the screening of serum antibodies, primarily IgG, for visceral *Leishmania* using the ELISA technique.

Summary

Visceral Leishmaniasis (VL) is a severe disease with high mortality, caused by parasite members of the *L. donovani* complex (1,2). The vector for transmission is the sand fly, whose carriers of infection are typically dogs (3-6). It is a disease endemic to many countries and is a serious problem in many developing nations, particularly with the increasing urbanization of populations (7). High incidence is encountered in parts of Latin America, East Africa, Middle East, India and China. It is endemic to countries bordering the Mediterranean such as Italy, Southern France, Spain, Portugal, and Northern Africa. In Southern Europe, VL has become the leading opportunistic infection in AIDS patients (8-15).

Diagnosis of acute VL is often attempted by aspiration of bone marrow for direct parasite identification. The procedure is invasive, painful, dangerous and has a low success rate due to the inability to always isolate parasites from the tissue. Alternatively, serodiagnosis is widely utilized since anti-leishmanial antibody titers are typically high during the acute disease phase. ELISA is the preferred laboratory test for serodiagnosis of VL, although indirect immunofluorescent antibody tests (IFAT) and direct agglutination tests (DAT), using whole parasites, are still widely used in conjunction with ELISA or alone (16-18).

Principle of Procedure

During the first incubation, the antibodies in the patient's serum bind to the antigens in the test well. The next incubation allows the antigen-antibody complex to bind to an enzyme complex. After washing the wells to remove unbound enzyme, a chromogen is added that develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

Reagents

Item	Description	Symbol
Test Strips	Microwells containing Leishmania antigens - 96 test wells in a test strip holder.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11 ml of anti-human Ig-Peroxidase (HRP) in a stabilizing buffer with Thimerosal.	CONJ
Positive Control	One (1) vial containing 2 ml of diluted surrogate positive in buffer with Thimerosal.	CONTROL +
Negative Control	One (1) vial containing 2 ml of diluted Leishmania-negative human sera in buffer with Thimerosal.	CONTROL −
Chromogen	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).	SUBS TMB
Wash Concentrate (20X)	Two (2) bottles 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

Precautions

Wash concentrate may show crystallization upon storage at 4 °C. Crystallization will disappear after diluting to working strength.
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.
Do not add azides to the samples or any of the reagents.
Controls and some reagents contain Thimerosal as a preservative.
Treat all sera as if capable of being infectious.
The negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. The positive control has not been tested for Hepatitis B surface antigen or for the antibody to HIV and should be treated as a potentially infectious agent. 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 Conditions

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

Preparation

Wash Buffer - Remove cap and add contents of bottle to 475 ml DI 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.

Collection And Preparation Of Serum

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

Materials Provided

- Visceral Leishmania Serology Microwell ELISA Kit

Materials Required But Not Provided

- Pipettes
- Squeeze bottle for washing strips
- DI water
- ELISA plate reader with a 450/620-650 nm filter (optionally, results can be read visually)
- Tubes for serum dilutions

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 100 ul 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 100 ul of Chromogen to every well.
9. Incubate at room temperature for 5 minutes.
10. Add 100 ul 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.

Test Limitations

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.

Interpretation of 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.

Performance Results

		IFA	
		+	-
New Life	+	30	10
	-	1	53

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

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 0.1 units. Should the values fall outside these ranges, the kit should not be used.

Troubleshooting

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

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