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Cysticercosis IgG (T. solium) ELISA Kit

Tsol-G-96

Intended Use

For the qualitative screening of serum IgG antibodies to *Taenia solium* using an Enzyme Linked Immunoabsorbant Assay (ELISA) technique.

Summary

Infection of the larval form (cysticerci) of *Taenia* in any tissue or organ is known as the disease cysticercosis. Many sites of infection have been documented but the central nervous system has been the most common. Presence of the cysticerci in the brain may cause increased cranial pressure, seizures and altered mental states. Any person with impaired CNS function should have the possibility of *T. solium* infection investigated.

The disease is acquired by ingestion of *T. solium* eggs from a number of different routes; including food contaminated with feces, unclean hands of *T. solium* infected workers, contaminated water or gastric reflux in tapeworm carriers.

Cysticercosis is rare in most industrialized nations but is endemic in developing areas such as Latin America, Asia and Africa. Most of the cases of cysticercosis in the United States are associated with immigrants from these countries.

Reliable diagnosis of cysticercosis requires multiple testing methods such as radiography and serology. Although use of cyst vesicular antigen has helped to increase its sensitivity and specificity, significant cross reactions with Echinococcosis occurs. If Echinococcus infection cannot be ruled out in the differential diagnosis, a positive sample should be confirmed by other means (i.e. immunoblot offered by the CDC) or by other non-serological means.

Principle of Procedure

The micro test wells are coated with *T. solium* cyst fluid 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 *T. solium* antigens - 96 test wells in a test strip holder.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11 ml of Protein A conjugated to peroxidase.	CONJ
Positive Control	One (1) vial containing 2 ml of a surrogate positive control.	CONTROL +
Negative Control	One (1) vial containing 2 ml of diluted negative human serum.	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

Do not use solutions if they precipitate or become cloudy. Wash concentrate may show crystallization upon storage at 2 – 8 ºC. Crystallization will disappear after dilution 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.
Treat all sera as if capable of being infectious. Negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. This product should be used under appropriate safety conditions that would be used for any potentially infectious agent.
Do not add azides to the samples or any of the reagents.

Storage Conditions

Reagents, strips and bottled components:
Store between 2 – 8 ºC.
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 of reagent grade water. Place diluted wash buffer into a squeeze bottle with a narrow tip opening.
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.

Collection and Preparation of Serum

Coagulate blood and remove serum. Freeze sample at -20 ºC or lower if not used immediately.
Do not heat inactivate serum and avoid repeated freezing and thawing of samples.
Test samples: Make a 1:64 dilution of patients' sera using the dilution buffer (e.g. 5 µl sera and 315 µl dilution buffer).

Procedure

Materials Provided
Cysticercosis Serology Microwell ELISA Kit

Materials Required But Not Provided
Pipettes
Squeeze bottle for washing strips (narrow tip is recommended)
Reagent grade water and graduated cylinder
Tubes for sample dilution
Absorbent paper

Suggested Materials
ELISA plate reader with a 450 nm and a 650 to 620 nm filter (optional if results are read visually)

Performance of Test

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Add 100 µl of the negative control to well #1, 100 µl of the positive control to well #2 and 100 µl of the diluted (1:64) test samples to the remaining wells.
   Note: Negative and positive controls are supplied prediluted. Do not dilute further.
3. Incubate at room temperature (15 to 25 ºC) for 10 minutes.
4. Shake out contents and wash 3 times with the diluted wash buffer.
5. Add 100 ul of Enzyme Conjugate to each well.
6. Incubate at room temperature for 5 minutes.
7. Shake out contents and wash 3 times with wash buffer. Slap wells against paper towels to remove excess moisture.
8. Add 100 ul of the Chromogen to every well.
9. Incubate at room temperature for 5 minutes.
10. Add 100 ul of the Stop Solution and mix by tapping strip holder.

*** Washings consist of vigorously filling each well to overflowing and decanting contents three (3) separate times. If using automated washers; add 1 minute dwell time between washings and increase number of washes from three to five.**

Reading of 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 450/650-620 nm.

Test Limitations

Serologic results are an aid in diagnosis but cannot be used as the sole method of diagnosis. Significant cross reactions with *Echinococcus* infections will occur in this assay. If *Echinococcus* infection cannot be ruled out in the differential diagnosis, a positive sample should be confirmed by other means (i.e. immunoblot offered by the CDC) or by other non-serological means.

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 0.1 OD units
Positive - 0.5 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 Results - ELISA Reader

Zero ELISA reader on air. Read all wells at 450/650 to 620 nm.
Positive - Absorbance reading equal to or greater than 0.2 OD units.
Negative - Absorbance reading less than 0.2 OD units.

A positive OD reading indicates that the patient may be infected by *T. solium* or a closely related organism (e.g.*Echinococcus*).
A negative 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 positive if the degree of color development is obvious and significant.

Expected Results

The number of individuals showing positive results can vary significantly between populations and geographic regions. If possible, each laboratory should establish an expected range for its patient population.

Performance Data

		Reference Method	
		+	-
New Life	+	72	2
	-	10	46

Sensitivity:
Thirty samples positive by immunoblot for cysticercosis infection were tested in the New Life ELISA kit; 26/30 samples were positive in the NLD ELISA giving a sensitivity of 87% versus the immunoblot.
Fifty-two samples positive by immunoblot for cysticercosis infection were tested in the New Life ELISA kit; 46/52 samples were positive in the New Life ELISA giving a sensitivity of 88% versus the immunoblot.
Specificity:
Forty-eight normal samples were tested in the NLD ELISA kit; 46/48 samples were negative in the New Life ELISA giving a specificity of 96%.

References

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2. Evans, C. et. Al., Controversies in the Management of Cysticercosis, *Emerging Infectious Diseases*, Vol. 3, No. 3, July-September 1997, pp.403-405
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