

Spirometra (Sparganosis) IgG ELISA Kit

Spiro-G-96

Intended Use

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

Summary

Adult members of the genus *Spirometra* live in the intestines of dogs and cats. Eggs are shed in the feces and embryonate in the environment where they go through a complicated life cycle. The cycle is completed when a predator (dog or cat) eats an infected second intermediate host. Humans cannot serve as definitive hosts for *Spirometra* spp. but serve as paratenic or second intermediate hosts and develop sparganosis. Humans acquire sparganosis by either drinking water contaminated with infected copepods or consuming the flesh of an under-cooked second intermediate or paratenic host. Spargana can live up to 20 years in the human host.

The genus *Spirometra* occurs worldwide in distribution, although most human cases of sparganosis are recorded from southeast Asian countries. Sparganosis is endemic in animals throughout North America, although human cases from this area are rare.

Migrating spargana cause various symptoms depending on the final location in the host. Spargana may locate anywhere, including subcutaneous tissue, breast, orbit, urinary tract, pleural cavity, lungs, abdominal viscera and the central nervous system. The migration in subcutaneous tissues is usually painless, but when spargana settle in the brain or spine a variety of neurological symptoms may occur, including weakness, headache, seizure, and abnormal skin sensations, such as numbness or tingling. If the inner ear is involved, the patient may experience vertigo or deafness. Occasionally, *Sparganum proliferum* can cause proliferative lesions in the infected tissue, with multiple plerocercoids present in a single site.

Principle of Procedure

The micro test wells are coated with recombinant *Spirometra erinaceieuropaei* cysteine proreainase antigen. During the first incubation with the diluted patients' sera, any antibodies that 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) and a substrate (hydrogen peroxide) are 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 <i>Spirometra</i> antigens – 96 test wells in a test strip holder.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11ml of Protein A conjugated to peroxidase.	CONJ
Positive Control	One (1) vial containing 2 ml of a surrogate positive.	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 °C. 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 azide to the samples or any of the reagents.

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 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 °C or lower if not used immediately.

Do not heat inactivate serum and avoid repeated freezing and thawing of samples.

Test samples: Make a 1:100 dilution of patient's sera using the dilution buffer (e.g. 5 µl sera and 495 µl dilution buffer).

Procedure

Materials Provided

Spirometra 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 (1:100) 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 °C) for 30 minutes.
4. Shake out contents and wash 3 times with the diluted wash buffer. Slap wells against paper towels to remove all the wash buffer.
5. Add 100 µl 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. Slap wells against paper towels to remove all of the wash buffer.
8. Add 100 µl of the Chromogen to every well.
9. Incubate at room temperature for 10 minutes.
10. Add 100 µl 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.

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 0.09 OD units
Positive -	0.3 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 the Test – ELISA Reader

1 – Calculate the average extinction value by taking the average OD value of the Negative Control.

2 – Add 0.200 to this average extinction value. This value is the cut-off value used in the Sample Index Calculation.

Example:

Negative Control 1 OD = 0.084

Negative Control 2 OD = 0.100

Average is $0.084 + 0.100 = 0.184 / 2 = 0.092$ = Average Extinction Value

Cut-off value is the Average Extinction Value + 0.200 (in this example $0.092 + 0.200 = 0.292$)

3 – Determine the Sample Index by dividing the patients OD value by the Cut-off value.

Example:

Patient OD value of 1.225

Cut-off value of 0.292

$1.225 / 0.292 = 4.19$

- 4 – Evaluate the Sample Index.
 Negative = less than 1.0 Sample Index
 Equivocal = 1.0 to 1.5
 Positive = greater than 1.5

Limitations of The Procedure

Diagnosis of infection should not be made solely 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.

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.

Performance Characteristics

		Reference Method*	
		+	-
Eiger ELISA	+	7	2
	-	0	94

Sensitivity of 100% (7/7)
 Specificity of 98% (94/96)

*Reference Method refers to the labs in-house assay.

References

1. CDC Website August 2024
2. WHO Website August 2024
3. Li Na Liu, et. Al. Characterization of *Spirometra erinaceieuropaei* Plerocercoid Cysteine Protease and Potential Application for Serodiagnosis of Sparganosis. PLOS Neglected Tropical Diseases, DOI:10.1371, June 5, 2015
4. Wei Liu, et. Al., Epidemiology, Diagnosis, and Prevention of Sparganosis in Asia. Animals (MDPI), 2022, 12, 1578