Angiostrongylus IgG ELISA Kit

Angio-G-96

Intended Use

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

Summary

Angiostrongylus cantonensis is a parasitic worm of rats. It is also called the rat lungworm. The adult form of the parasite is found only in rodents. Infected rats pass larvae of the parasite in their feces. Snails and slugs get infected by ingesting the larvae. These larvae mature in snails and slugs but do not become adult worms. The life cycle is completed when rats eat infected snails or slugs and the larvae further mature to become adult worms.

People can get infected by eating raw or undercooked snails or slugs that are infected with this parasite. In some cultures, snails are commonly eaten. Some children, in particular, have gotten infected by swallowing snails/slugs "on a dare." People also can get infected by accident, by eating raw produce (such as lettuce) that contains a small snail or slug or part of one.

Certain animals such freshwater shrimp, crabs, or frogs, have been found to be infected with larvae of the parasite. It is possible that eating undercooked or raw animals that are infected could result in people becoming infected, though the evidence for this is not as clear as for eating infected snails and slugs. Of note, fish do not spread this parasite.

Principle of Procedure

The micro test wells are coated with *Angiostrongylus* 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 *Angiostrongylus* 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 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 azide 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 1:100 dilution of patient's sera using the dilution buffer (e.g. 5 µl sera and 495 µl dilution buffer).

Procedure

Materials Provided
Angiostrongylus 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 ºC) 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.**

For Automated Use: Decrease run times to 7-4-3 minute steps.

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 diving 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*	
		+	-
NLD ELISA	+	9	2
	-	0	89

Sensitivity of 100% (9/9)
Specificity of 98% (89/91)

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

References

1. CDC Website May 2023
2. WHO Website May 2023
3. Meesing et. Al, Transmission sources and severe rat lung worm diseases in travelers: a scoping review, Trop Dis Travel Med and Vaccines, 9: (2023)
4. Eamsobhana, P, Yong, H. S., Immunological diagnosis of human angiostrongyliasis due to Angiostrongylus cantonensis (Nematoda: Angiostrongylidae), Intl J Infect Dis 13, 425-431, (2009)
5. Somboonpatarakun et. Al., Application of Recombinant Angiostrongylus cantonensis Galectin-2 Protein for Serodiagnosis of Human Angiostrongyliasis by Immunoblotting. Am J Trop Med Hyg, 101(4), 851-858 (2019)