Ancylostoma IgG ELISA Kit

Ancylo-G-96

Intended Use

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

Summary

Ascaris lumbricoides (*Ascaris* or roundworm), *Ancylostoma duodenale* (hookworm), *Necator americanus* (hookworm), and *Trichuris trichiura* (whipworm) are helminths (parasitic worms) that infect the intestine. Due to the role of contaminated soil in their transmission, this group of nematode worms are known as soil-transmitted helminths (STH). *Strongyloides stercoralis* (threadworm) is sometimes included in the STH.

STH are transmitted through ingestion of the tiny, infectious eggs of Ascaris, whipworm, and some hookworm, and through skin transmission for hookworm. People of all ages can become infected. Adult female worms produce thousands of eggs daily that are passed in feces and, if conditions allow, deposited in soil. Once in soil, infective larvae of *Ascaris* and whipworms develop in the fertile eggs and, if ingested by a human host, hatch and develop into adult worms over several months. Hookworm eggs are not infective—the eggs hatch and release larvae that must mature in soil before they become infective. Hookworm infection usually occurs when larvae penetrate the skin of people walking barefoot on contaminated soil; *Ancylostoma duodenale* also can be transmitted when larvae are ingested. Occasionally, human infection with *Ascaris suum* (pig roundworm) can occur due to ingestion of infectious eggs shed in pig feces.

Globally, ≈2 billion people are infected with ≥1 STH, which together account for most parasitic disease burden worldwide. STH have widespread global distribution and are endemic in countries with tropical or subtropical climates and where sanitation is poor, human feces are used as fertilizer (“night soil”), or water supplies are contaminated. Although all travelers to endemic countries have some risk for STH infection, risk increases for long-term travelers and expatriates going to countries with poor general sanitation. Travelers can minimize risk by taking preventive measures.

Historically, STH infections were common in people living in US states where warm, moist climate and lack of sanitation enabled transmission; current prevalence of infection in those areas is unknown. Most reported infections in the United States are among immigrant and refugee populations. Since the introduction of predeparture treatment, stool testing for STH is unnecessary for most refugees. Because Ascaris, whipworm, and hookworm do not multiply in hosts (as opposed to threadworm), reinfection occurs only as a result of additional exposure to the infective-stage larvae.

Principle of Procedure

The micro test wells are coated with *Ancylostoma* 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 *Ancylostoma* 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

Ancylostoma 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.**

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*	
		+	-
Eiger ELISA	+	8	1
	-	0	92

Sensitivity of 100% (8/8)
Specificity of 99% (92/93)

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

References

1 – CDC Website (www.cdc.gov)

2 – WHO Website (www.who.org)

3 – Parasitology (Microbeonline.com); Lab Diagnosis of Intestinal Parasite Infections; A. Tankeshwar; 4 Oct 2022

4 – StatPearls On-Line; National Library of Medicine; Ancylostoma; M. Aziz & K. Ramphul; 10 April 2022