Ascaris Serum IgG Antibody ELISA Test Kit 96 Wells

Ascaris-G-96

Intended Use

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

Summary

Ascaris lumbricoides has probably been infecting humans for thousands of years.[1] It is the most common nematode parasite infecting humans and over 1 billion people are believed to be infected.[3] Children who live in moist, warm climates are the most at risk to become infected.[1,3] Ingesting embryonated eggs from contaminated soil is the primary means of infection.[1] The eggs will hatch in either the stomach or small intestine where the larvae penetrate through the intestine wall.[1-3] Larvae are carried to the heart and then to the lungs, where they stay for approximately 10 days. Larvae will then go into the alveoli and migrate via the bronchi to the trachea and pharynx. The larvae are coughed up, swallowed, and returned to the intestine where they mature and mate, eventually producing eggs.[1,3] This process occurs over 8 – 12 weeks. Eggs will get passed into the environment via feces. Fertilized eggs will become infective within 2 weeks if they are kept in warm, moist soil. Adult worms usually live for about 1 year. Females are 20 – 35 cm long and can produce 200,000 eggs per day where as males are 15 – 31 cm long and have a curved posterior end.[1-3]

Pathogenisis in humans from *Ascaris* can be cause by the host's immune response, effects of larvae migration, mechanical effects of adult worms, and also nutritional deficiencies caused by the adult worms being present in the body. There are usually no symptoms involved with the initial passage of larvae through the liver and lungs unless the number of worms is high. Bronchial epithelium damage may result from larvae exiting lung tissue.[1] When successive migrations of larvae occur, more intense tissue reactions may be observed. This could be accompanied by a dry or productive cough, fever, transient eosinophilia, dyspnea, and a chest x-ray that resembles viral pneumonia; this condition being referred to as *Ascaris* pneumonitis. The presence of adult worms in the intestine usually shows no symptoms unless the number of worms present is high. However, even a single worm can cause damage due to the worms tendency to migrate.[1,2] Certain stimuli may result in migration such as fever, general anesthesia, as well as other abnormal body conditions. This can result in entry into the bile duct, liver or other small spaces as well as intestinal blockage.[1] Worms have been known to spontaneously migrate out of the body through the mouth, nose or anus.[1,2] Eighty-five percent of humans infected with *Ascaris* will exhibit no symptoms.[3]

The primary means of preventing the spread of *Ascaris* infection is through the use of appropriate sanitation facilities and practices, such as frequent washing of the hands. The use of human feces, known as night soil, as fertilizer should be recognized as potentially hazardous.[1,3] Any fruits or vegetables that are grown using night soil cannot be eaten raw. Even after fields using night soil have been processed and treated, *Ascaris* eggs may still remain viable and infective.[1]

Principle of Procedure

The micro test wells are coated with *Ascaris* 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 *Ascaris* 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 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 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
Ascaris 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 (or two drops) 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 10 minutes.
4. Shake out contents and wash 3 times with the diluted wash buffer.
5. Add 2 drops 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 all of the wash buffer.
8. Add 2 drops of the Chromogen to every well.
9. Incubate at room temperature for 5 minutes.
10. Add 2 drops 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 Results - ELISA Reader

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

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.

References

1. Bruckner, D., Garcia, L. Diagnostic Medical Parasitology. 2nd Edition. American Society for Microbiology, 1993. pp. 184-192.
2. Murray, P. et al. Manual of Clinical Microbiology. 7th Edition. American Society for Microbiology, 1999. p. 1424.
3. Bogitsh, B., Cheng, T. Human Parasitology. Saunders College Publishing, 1990. pp. 321-325.