Ascaris IgM ELISA Kit

Ascaris-M-96

Intended Use

The *Ascaris* ELISA test is a semi-quantitative enzyme immunoassay for the detection of IgM antibodies to *Ascaris*, in samples of human serum or plasma. This test is intended to be performed by trained medical technologists only.

Summary and Explanation

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]

Assay Principle

The microwells are coated with *Ascaris* 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 *Ascaris* antigens - 96 test wells in a test strip holder.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11 ml of anti-human IgM (μ chain specific) conjugated to peroxidase.	CONJ
Positive Control	One (1) vial containing 2 ml of diluted surrogate positive control.	CONTROL +
Negative Control	One (1) vial containing 2 ml of diluted human sera.	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
RF Absorbent	One (1) bottle containing anti-human IgG	SOLN

Statement of Warnings

- **Do not deviate from the specified procedures when performing this assay**. All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- For *In Vitro* Diagnostic Use Only.
- Do not interchange reagents between kits with different lot numbers.
- Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.
- Unused microwells should be stored in the desiccated pouch to protect them from moisture.
- Do not use solutions if they precipitate or become cloudy.
  Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
- Do not add azides to the samples or any of the reagents.
- Controls and some reagents contain Thimerosal as a preservative, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.
- 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 reagents and samples as potentially infectious materials. Negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV be required test methods. Use care to prevent aerosols and decontaminate any spills of samples.
- Stop solution is a 5% solution of phosphoric acid in water. If spilled on the skin, wash with copious amounts of water. If acid gets into the eyes, wash with copious amounts of water and seek medical attention.

Storage

- Reagents, strips and bottled components should be stored at 2-8 °C
- Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25 °C)

Preparation

- Before use, bring all reagents and samples to room temperature (15-25 °C) and mix.
- (20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature and mixed. ***Ensure that (20X) Wash Concentrate is completely in solution before diluting to working concentration.*** To dilute (20X) wash concentrate to working dilution, remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

Specimen Collection And Handling

Serum or plasma may be stored at 2-8 ºC for up to five days. Sample may be frozen below -20 ºC for extended periods. Freezing whole blood samples is not advised. Do not heat inactivate samples and avoid repeated freezing and thawing of samples.

Sample Preparation

Dilute patient sera 1:100 using the Dilution Buffer (e.g. 5 µl sera and 500 µl dilution buffer). Then add 100 µl of this diluted sera to a tube containing 40 µl of RF Absorbent. Mix and allow to incubate at room temperature for approximately 10 minutes.

Procedure

Materials Provided

Ascaris IgM ELISA Kit

Materials Required But Not Provided

- Micropipette
- Reagent grade (DI) water
- Graduated Cylinder
- Timer
- Tubes for serum dilution

Suggested Materials

ELISA plate reader with a 450 nm and a 620 - 650 nm filter

Proper Temperature

All incubations are at room temperature (15-25 °C)

Test Procedure

Notes:

- Ensure all samples and reagents are at room temperature (15-25°C)
- Negative and positive controls are supplied pre-diluted. DO NOT dilute further.

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Dilute patient sera as described above in Sample Preparation Section.
3. Add **100 µl** of the negative control to well #1 and **100 µl** of the positive control to well #2 and **100 µl** of the diluted and RF absorbed test samples to the remaining wells.
4. Incubate at room temperature for **30 minutes**, then wash.*
5. Add **100 µl** of Enzyme Conjugate to each well.
6. Incubate at room temperature for **15 minutes**, then wash.*
7. Add **100 µl** of the Chromogen to each well.
8. Incubate at room temperature for **10 minutes**.
9. Add **100 µl** of the Stop Solution to each well. Mix contents by gently tapping the side of the strip holder.
10. Read within one hour of adding Stop Solution.

*** Washings consist of 5 washings of 300 µl per well for each step with a 30 second dwell time for each wash set. If possible, slap out excessive wash buffer from the wells against absorbent toweling before addition of the next reagent.**

Proper and thorough washing is key to obtaining accurate and reproducible results.

Reading Results

ELISA Reader: Zero reader on air. Set for bichromatic readings at 450/620-650 nm.

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 0.2 OD units
Positive - 0.5 OD units and above

Interpretation of the Test – ELISA Reader

Non-Reactive:	OD values less then 0.25
Equivocal:	OD values of 0.25 to 0.45
Reactive:	OD values above 0.45

Limitations of The Procedure

Diagnosis of *Ascaris* infection should not be made solely based on results of the ELISA *Ascaris* 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.

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.