

**AccuDiag™**  
**Ascaris IgG**  
**ELISA Kit**

REF 8212-35



Test	Ascaris IgG ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Indirect; Antigen Coated Plate
Detection Range	Qualitative: Positive, Negative
Sample	5 µL
Total Time	~ 20 min.
Shelf Life	12 Months from the manufacturing date
Specificity	96%
Sensitivity	100%

**INTENDED USE**

The Diagnostic Automation *Ascaris* ELISA test for the qualitative screening of serum IgG antibodies to *Ascaris* using an Enzyme Linked Immunoabsorbant Assay (ELISA) technique.

**SUMMARY AND EXPLANATION**

*Ascaris lumbricoides* has probably been infecting humans for thousands of years.<sup>1</sup> It is the most common nematode parasite infecting humans and over 1 billion people are believed to be infected.<sup>3</sup> Children who live in moist, warm climates are the most at risk to become infected.<sup>1,3</sup> Ingesting embryonated eggs from contaminated soil is the primary means of infection.<sup>1</sup> The eggs will hatch in either the stomach or small intestine where the larvae penetrate through the intestine wall.<sup>1-3</sup> 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.<sup>1,3</sup> 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.<sup>1-3</sup>

Pathogenesis in humans from *Ascaris* can be caused 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.<sup>1</sup> 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.<sup>1,2</sup> 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.<sup>1</sup> Worms have been known to spontaneously migrate out of the body through the mouth, nose or anus.<sup>1,2</sup> Eighty-five percent of humans infected with *Ascaris* will exhibit no symptoms.<sup>3</sup>

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.<sup>1,3</sup> 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.<sup>1</sup>

**TEST PRINCIPLE**

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.

**SPECIMEN COLLECTION AND PREPARATION**

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).

**MATERIALS AND COMPONENTS**

**Materials provided with the test kits**

- Plate:** Microwells containing *Ascaris* antigens - 96 test wells in a test strip holder.
- Enzyme Conjugate:** One (1) bottle containing 11 ml of Protein A conjugated to peroxidase.
- Positive Control:** One (1) vial containing 1 ml of diluted positive rabbit sera.
- Negative Control:** One (1) vial containing 1 ml of diluted negative human sera.
- Chromogen:** One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
- Wash Concentrate 20X:** One (1) bottle containing 25 ml of concentrated buffer and surfactant.
- Dilution Buffer:** Two (2) bottles containing 30 ml of buffered protein solution.
- Stop Solution:** One (1) bottle containing 11 ml of 1 M phosphoric acid.

**Materials required but not provided**

- Micropipette
- Squeeze bottle for washing strips (narrow tip is recommended)
- Reagent grade water and graduated cylinder
- Tubes for sample dilution
- Absorbent paper
- ELISA plate reader with a 450 nm and a 620-650 nm filter (optional if results are read visually).

**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.

**ASSAY PROCEDURE**

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 °C) 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.

**\*CAUTION! WHEN USING AN AUTOMATED OR SEMI-AUTOMATED WASHING SYSTEM THE FOLLOWING MUST BE FOLLOWED. FAILURE TO DO SO WILL RESULT IN INADEQUATE WASHING OF THE WELLS AND MAY LEAD TO FALSE POSITIVE RESULTS!**

**Washing Procedure for Auto and Semi-Automated Washers**

- Perform five (5) washes per step instead of three
- Set machine to "soak" for one minute between each step
- After each set of washings, slap wells against an absorbent towel.

**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 450/620-650 nm.

**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**

Zero ELISA reader on air. Read all wells at 450/650 to 620 nm.

**Positive** - Absorbance reading greater than 0.1 OD units.

**Negative** - Absorbance reading less than 0.1 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.

**PERFORMANCE CHARACTERISTICS**

		Reference Method *	
		+	-
Diagnostic Automation, Inc.	+	12	2
	-	0	54

Sensitivity- 100%

Specificity-96

\*Reference Laboratory

**EXPECTED VALUES**

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.

**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

**LIMITATIONS OF PROCEDURE**

Serologic results are an aid in diagnosis but cannot be used as the sole method of diagnosis.

**PRECAUTIONS**

1. 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.
2. 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.
3. 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.
4. Do not add azide to the samples or any of the reagents.

**STORAGE**

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

**REFERENCES**

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

**ISO 13485**  
**ISO 9001**



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