AccuDiag™ Toxocara IgG ELISA Kit

Cat # 8206-35

IVD See external Label 2°C 8°C 96 Tests

Test	Toxocara IgG ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Semi-Quantitative : Positive, Negative
Sample	5 µL serum
Total Time	~ 20 min.
Shelf Life	12 Months from the manufacturing date
Specificity	100%
Sensitivity	100%

INTENDED USE

The Toxocara ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgG antibodies to Toxocara in human serum.

SUMMARY AND EXPLANATION

Infected humans cannot pass the Toxocara infection to other humans. Causes of the toxocariasis infection stem from swallowing dirt containing Toxocara eggs from animal feces. The infection is caused by roundworm larvae of two species of Toxocara - (T. canis) from dogs and (T.cati) from cats. Toxocara is not contagious from one human to another, and because the eggs become hatched after 2 to 5 weeks, humans do not acquire this infection from fresh feces.

Toxocara larva migrans has been labeled the second most common parasitic worm in many countries throughout the world. Toxocara infections migrate to different parts of the body and consequently can develop into varied symptoms. One common type is OLM (ocular larval migrans), where the larva migrates to the eye. Symptoms range from asymptomatic to serious eye infections. Another type of manifestation is VLM (visceral larval migrans), where the body's organs, or central nervous system, are infected. Patients with VLM may experience serious fatal conditions, develop a blood disorder, or show no signs of symptoms at all.

TEST PRINCIPLE

The principle of the Toxocara ELISA test is a three-incubation process whereby the first incubation involves the coating of the wells with an excretory/secretoroy antigen from the Toxocara larvae. During this step, using the diluted patients' sera, any antibodies that are reactive with the antigen, will bind to the wells. Next, the wells must be washed to remove test sample. At this point Enzyme Conjugate is added. During this second incubation, the Enzyme Conjugate will bind to any antibodies present. Before the third incubation step, additional washings are necessary. Then a chromogen (tetramethylbenzidine or TMB) is added. With the presence of Enzyme Conjugate and the peroxidase causing the consumption of peroxide, the chromogen changes to a blue color. The blue color turns to a bright yellow color after the addition of the stop solution, which ends the reaction. ELISA readers can be used to obtain results, or the reaction can be ready visually.

SPECIMEN COLLECTION AND PREPARATION

Serological specimens should be collected under aseptic conditions. Coagulate blood and remove serum. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2 – 8 ºC if it is to be analyzed within a few days. Serum may be held for 3-6 months by storage at -20 ºC or lower. Do not heat inactivate serum and avoid repeated freezing and thawing of samples.
Test samples: Make a 1:64 dilution of patients' sera using the dilution buffer (e.g. 5 µl sera and 315 µl dilution buffer).

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Plate***:* Microwells containing *Toxocara* antigens – 96 test wells in a test strip holder.
2. **Enzyme Conjugate**: One (1) bottle containing 11 ml of Protein A conjugated to peroxidase.
3. **Positive Control**: One (1) vial containing 1 ml of diluted positive rabbit serum.
4. **Negative Control:** One (1) vial containing 1 ml of diluted negative human serum.
5. **TMB Substrate Solution:** One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
6. **Wash Concentrate 20X**: One (1) bottle containing 25 ml of concentrated buffer and surfactant.
7. **Dilution Buffer:** One (1) or Two (2) bottles containing 30 ml of buffered protein solution.
8. **Stop Solution**: One (1) bottle containing 11 ml of 0.73 M phosphoric acid.

Materials required but not provided

1. Pipettes
2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Reagent grade water and graduated cylinder
4. Tubes for sample dilution
5. Absorbent paper
6. Absorbent paper
7. 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 (1:64) 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. Add100 µl 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 plates against paper toweling to remove excess moisture.

8. Add 100 µl of the Chromogen to every well.
9. Incubate at room temperature for 5 minutes.
10. Add 100 µl of the Stop Solution and mix by tapping strip holder.

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

Zero ELISA reader on air. Read all wells at 450/650-620 nm.
Positive - Absorbance reading equal to or greater than 0.3 OD units.
Negative - Absorbance reading less than 0.3 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 significant and obvious.\

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 0.3 OD units
Positive - 0.5 OD units and above

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.

PERFORMANCE CHARACTERISTICS

		Reference Method *	
		+	-
Diagnostic Automation,Inc.	+	22	0
	-	0	65

Specificity of 100% (22/22)
Sensitivity of 100% (65/65)
*Reference Method refers to a commercially available ELISA.

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 ºC. 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 azides to the samples or any of the reagents.

STORAGE

Reagents, strips and bottled components:
Store between 2 – 8 ºC.
Squeeze bottle containing diluted wash buffer may be stored at room temperature.

REFERENCES

1. Glickman, L., P. Schantz, and R. Grieve. "Toxocariasis". Immunodiagnosis of
2. Parasitic Diseases. Vol.1, Helminthic Diseases. Ed. Walls and Schantz.
3. Academic
4. Press, 1986. pp. 201-231.
5. Schantz, P. "Toxocara Larva Migrans Now." Am J Trop Med Hyg. Vol. 41 (Sup 3), 1989, pp. 21-34.
6. Jacquier, P. et. al. "Immunodiagnosis of Toxocarosis in Humans: Evaluation of a New Enzyme-Linked Immunosorbent Assay Kit." J Clin Micro. Vol. 29 #9, Sept. 1991, pp. 1831-1835.
7. Carlier, Y. et. al. "The Use of an Excretory-Secretory Antigen for an ELISA Specific 39-42.
8. Brunello, F., P. Falagiani, and C. Genchi. "Enzyme Immunoassay (ELISA) for the Detection of Specific IgG Antibodies to Toxocara canis ES Antigens." Boll. Ist. sieroter. Milan. Vol. 65 #1, 1986, pp. 54-60.
9. Josephson, S.L., "Toxocariasis", Laboratory Diagnosis of Infectious Diseases – Principles and Practices, Vol. 1, 1988, pp. 993-997.

ISO 13485
ISO 9001

Diagnostic Automation/Cortez Diagnostics, Inc.
23961 Craftsman Road, Suite E/F,
Calabasas, California 91302 USA

Date Adopted	Cat # 8206-35
2013-09-18	AccuDiag™- Toxocara IgG ELISA -2013
EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu

Revision C Date: 12-11-2013