DRG® Toxocara canis ELISA (EIA-3518)

Revised 12 Feb. 2011 rm (Vers. 6.1)

IVD

Please use only the valid version of the package insert provided with the kit.

Intended Use

For the qualitative screening of serum IgG antibodies to Toxocara using an Enzyme-Linked Immunosorbent Assay (ELISA) technique.

For In Vitro Use.

Summary

Toxocariasis is the infection caused by roundworm of the genus Toxocara (usually T. canis, rarely T. cati) and is acquired by ingesting soil contaminated with embryonated eggs from an animal's feces. These eggs become embryonated after 2 to 5 weeks after being passed by the animal. Thus, human infection does not occur by contact with fresh feces. In addition, infected humans cannot pass the infection to other humans. 6

The disease manifests itself as visceral larval migrans (VLM) and ocular larval migrans (OLM). Signs and symptoms of VLM may vary from an asymptomatic state with mild eosinophilia to a severe and potentially fatal disorder. Patients with OLM also vary widely in presentation, from acute lesions in the eye to asymptomatic infections. Toxocara larva migrans is believed to be the second most common helminth infection in developed countries.1,2

There is no definitive method to diagnose Toxocara infections, thus true sensitivity and specificity of serologic tests cannot be accurately determined. The diagnosis is further complicated by the fact that the antibody response varies depending on worm burden and location. However, numerous studies have shown that immunoassays using a purified excretory antigen from the larval stage, as in this ELISA, have shown dramatically improved sensitivities and specificities when compared to assays using crude antigens.1-6

Principle of Procedure

The micro test wells are coated with an excretory/secretory antigen from the Toxocara larvae. 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 *Toxocara* antigens – 96 test wells in a test strip holder.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11 ml of Protein A conjugated to peroxidase.	CONJ
Positive Control	One (1) vial containing 1 ml of diluted positive rabbit serum.	CONTROL +
Negative Control	One (1) vial containing 1 ml of diluted negative human serum.	CONTROL -
Chromogen	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).	SUBS TMB
Wash Concentrate (20X)	One (1) bottle containing 25 ml of concentrated buffer and surfactant.	WASH BUF
Dilution Buffer	Two (2) bottles containing 30 ml of buffered protein solution.	SPECIM DIL
Stop Solution	One (1) bottle containing 11 ml of 0.73 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 azides 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

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°C if it is to be analyzed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided. 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).

Procedure

Materials Provided

- Toxocara 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 (1:64) 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. 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 plates against paper toweling to remove excess moisture.
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.

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 0.3 OD units
Positive - 0.5 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

Interpretation of Results - ELISA Reader

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.

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.

Performance Data

Study #1 – Canadian Reference Center
Compared DRG ELISA to another commercial ELISA. Found concordance of 84% (n=82).

Study #2 – Mayo Clinic

		Reference Method	
		+	-
DRG	+	21	1
	-	3	14

Sensitivity of 87.5% (21/24)

Specificity of 93.3% (14/15)

References

1. Glickman, L., P. Schantz, and R. Grieve. "Toxocariasis". Immunodiagnosis of Parasitic Diseases. Vol.1, Helminthic Diseases. Ed. Walls and Schantz. Academic Press, 1986. pp. 201-231.
2. Schantz, P. "Toxocara Larva Migrans Now." Am J Trop Med Hyg. Vol. 41 (Sup 3), 1989, pp. 21-34.
3. 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.
4. Carlier, Y. et. al. "The Use of an Excretory-Secretory Antigen for an ELISA Specific Serodiagnosis of Visceral Larva Migrans." Biomedicine. Vol. 36, 1982, pp. 39-42.
5. 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.
6. Josephson, S.L., "Toxocariasis", Laboratory Diagnosis of Infectious Diseases – Principles and Practices, Vol. 1, 1988, pp. 993-997.

Version 12/28/10~rm