Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ Toxocara IgG ELISA Kit

REF 8206-35

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

Test	Toxocara IgG ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Qualitative Positive; Negative Control
Sample	5 µL serum/plasma
Total Time	~ 20 min.
Shelf Life	12 Months from the manufacturing date
Specificity	93.3%
Sensitivity	87.5%

INTENDED USE

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

SUMMARY AND EXPLANATION

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]

TEST PRINCIPLE

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.

SPECIMEN COLLECTION AND PREPARATION

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.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. Test Strip: 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: 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: Two (2) bottles containing 30 ml of buffered protein solution.
8. Stop Solution: One (1) bottle containing 11 ml of 1 M phosphoric acid.

Materials required but not provided

9. Micropipette
10. Squeeze bottle for washing strips (narrow tip is recommended)
11. Reagent grade (DI) water
12. Graduated Cylinder
13. Sample Dilution Tubes
14. Absorbent paper

Suggested Materials

ELISA plate reader with a 450 nm and a 620-650 nm filter (optional if results are read visually)

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.

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ASSAY PROCEDURE

Notes:

- Ensure all samples and reagents are at room temperature (15-25 °C)
- When running the assay, try to avoid the formation of bubbles in the wells. Bubbles may affect overall performance and reading of end results. Slapping the wells out on a clean absorbent towel after each step should help to minimize bubbles in the wells.
- 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 1:64 in Dilution Buffer (e.g. 5 µl sera and 315 µl dilution buffer). Add **100 µl** 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.
3. Incubate at room temperature (15 to 25 ºC) for **10 minutes**, then wash*. After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer.
4. Add **100 µl** of Enzyme Conjugate to each well.
5. Incubate at room temperature for **5 minutes**, then wash*. After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer.
6. Add **100 µl** of the Chromogen to every well.
7. Incubate at room temperature for **5 minutes**.
8. Add **100 µl** of the Stop Solution to each well. Mix wells by gently tapping the side of the strip holder with index finger for approximately **15 seconds**.

* Washings consist of vigorously filling each well to overflowing and decanting contents three (3) separate times. When possible, avoid formation of bubbles in the wells as this may affect the end results.

***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 450/620-650 nm.
Positive - Absorbance reading greater than or equal to 0.3 OD units.
Negative - Absorbance reading less than 0.3 OD units.

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 antibody positive subjects in a population depends on two factors: disease prevalence and clinical criteria used to select the tested population. Because very few positives should be seen in a randomly screened population in a non-endemic area, most serology tests are not specific enough to screen non-endemic populations. Even in an endemic region, serology screening often yields many false positives if used to randomly screen patients. Serology tests are useful to test patients in an endemic region with signs and symptoms consistent with the disease.

PERFORMANCE CHARACTERISTICS

Study #1 – Canadian Reference Center
Compared Diagnostic Automation, Inc. ELISA kit to another commercial ELISA. Found concordance of 84% (n=82).

Study #2 – Mayo Clinic

		Reference Method *	
		+	-
Diagnostic Automation,Inc.	+	21	1
	-	3	14

Specificity of 87.5% (21/24)
Sensitivity of 93.3% (14/15)
*Reference Method refers to a commercially available ELISA.

LIMITATIONS OF PROCEDURE

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

PRECAUTIONS

- **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.

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- 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)

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