Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

CE

AccuDiag™ Strongyloides IgG ELISA Kit

Cat # 8319-35

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

Test	Strongyloides 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	100%
Sensitivity	100%

INTENDED USE

The Strongyloides ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of IgG antibodies to Strongyloides stercoralis in serum and plasma. The Strongyloides ELISA test is for use only by a laboratory.

SUMMARY AND EXPLANATION

The parasite Strongyloides stercoralis is the causative agent for Strongyloidiasis disease. These parasites are endemic to tropical and semi-tropical environs, but are also found in various regions throughout the world. The parasite is known as an intestinal nematode which invades the body through exposed skin. Intestinal problems occur, such as diarrhea, but in more severe cases where the patient's immune system may be compromised, meningitis or septic shock may develop.

The Strongyloides ELISA kit is an especially appropriate serological test to use if the infection occurs outside the intestine where it is important to keep the disease isolated from other conditions, such as hemotologic malignancies.

TEST PRINCIPLE

The principle of the Strongyloides ELISA test is a three-incubation process whereby the first incubation involves the coating of the wells with Strongyloides antigen. During this step, any antibodies that are reactive with the antigen, will bind to the coated 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, more washings are necessary. Then, the 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 results may be read visually.

SPECIMEN COLLECTION AND PREPARATION

Serological specimens should be collected under aseptic conditions. 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 to 6 months by storage at -20 ºC or lower.
Pipemic 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 patient's 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 Strongyloides 3 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. **Milk Dilution Buffer:** 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. Micropipette
2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Reagent grade (DI) water and graduated cylinder
4. Tubes for sample dilution
5. Absorbent paper
6. 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.

ASSAY PROCEDURE

- 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 (or two drops) of the negative control well # 1, 100 µl of the positive control 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 100µl 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 100µl of the Chromogen to every well.
7. Incubate at room temperature for **5** minutes.
8. Add 100µl 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.

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.

Zero ELISA reader on air. Read all wells at 450/620-650 nm.
Positive - Absorbance reading greater than 0.2 OD units.
Negative - Absorbance reading less than 0.2 OD units.

A positive OD reading indicates that the patient may be infected by *Strongyloides*.
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.

Visual INTERPRETATION OF RESULT - VISUAL

Compare results to the controls. A sample should be interpreted as positive if the degree of color is significant and obvious.

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 0.2 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.

PERFORMANCE CHARACTERISTICS

		Reference Method *	
		+	-
Diagnostic Automation,Inc.	+	14	0
	-	0	14

Specificity: 14/14 = 100%
Sensitivity: 14/14 = 100%

*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 deviate from the specified procedures when performing this assay. All specimen dilutions, incubations 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.
2. For in Vitro Diagnostic Use Only.
3. Do not interchange reagents between kits with different lot numbers.
4. 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.
5. Unused microwells should be stored in the desiccated pouch to protect them from moisture.
6. Do not use solutions if they precipitate or become cloudy.
7. Exception: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
8. Do not add azides to the samples or any of the reagents.
9. 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.
10. 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.
11. 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.
12. 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.
13. Persons who are color blind or visually impaired may not be able to read the test visually and should use spectrophotometric readings to interpret results.

STORAGE

Reagents, strips and bottled components:
Store between 2 – 8 ºC.
Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25ºC)

REFERENCES

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ISO 9001

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