IMMUNOSPEC CORPORATION

7018 Owensmouth Ave. Suite 103
Canoga Park, CA, 91303
Phone: 818-710-1281
Fax: 818-936-0121
Email:Info@immunospec.com
www.immunospec.com

CE

Strongyloides

REF

Catalog No:E21-182

FOR RESEARCH USE ONLY

Intended Use

For the qualitative screening of serum IgG antibodies to Strongyloides stercoralis using an Enzyme Linked Immunoabsorbant Assay (ELISA) technique.

Summary

Strongyloidiasis is the disease caused by the Strongyloides stercoralis parasite. This organism is an intestinal nematode with worldwide distribution, but is especially common in tropical and subtropical regions. The disease usually manifests as intestinal symptoms (mild diarrhea). In a minority of cases, the organism will become extra-intestinal and may lead to septic shock and meningitis.
Serological tests are useful in detecting infection by Strongyloides if the organism goes extra-intestinal and in excluding the organism from the diagnosis of other disorders (especially hematologic malignancies). Strongyloides infected patients are particularly at risk for severe complications if they are also immunocompromised.

Principle of Procedure

The micro test wells are coated with Strongyloides antigen. 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.

Item	Description
Test Strips	Microwells containing Strongyloides 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 serum.
Negative Control	One (1) vial containing 1 ml of diluted negative human serum.
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 0.73 M phosphoric acid.

Statement Of Warnings

- **For Research Use Only.**
- **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.
- 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.
- 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.
- 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 should be stored at 2-8 °C
- Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25 °C)

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.

Collection and Preparation Of Serum

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 °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 patient's sera using the dilution buffer (e.g. 5 µl sera and 315 µl dilution buffer).

Procedure

Materials Provided
Strongyloides Serology Microwell ELISA Kit

Materials Required But Not Provided

- Micropipette
- Squeeze bottle for washing strips (narrow tip is recommended)
- Reagent grade (DI) water
- Graduated Cylinder
- Sample Dilution Tubes
- Absorbent paper

Suggested Materials

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

Performance Of Test

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 (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.
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 2 drops 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 2 drops of the Chromogen to every well.
7. Incubate at room temperature for 5 minutes.
8. Add 2 drops 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.

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 450/620-650 nm.

Test Limitations

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

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.

References

1. Schaffel, R. et. al. The Value of An Immunoenzymatic Test for the Diagnosis of Strongyloidiasis in Patients Immunosuppressed by Hematologic Malignancies. A, J Trop Med Hyg. #65(4) 2001. pp. 346-350.
2. Libman, M. et. al. Screening for Schistosomiasis, Filariasis and Strongyloidiasis Among Expatriates Returning from the Tropics. Clin Infect Dis. #17, 1993 pp.353-359
3. Loutfy, M. et. al.. Serology and Eosinophil Count in the Diagnosis and Management of Strongyloides in a Non-endemic Area. Am J Trop Med Hyg. #66(6). 2002 pp.749-752
4. Siddiqui, A. and Berk, S. Diagnosis of Strongyloides stercoralis Infection, CID #33 2001 pp. 1040-1047

EC REP **European Authorized Representative:**
CEpartner4U , Esdoornlaan 13, 3951DB Maarn
. The Netherlands. Tel.: +31 (0)6.516.536.26

Manufacturer:
IMMUNOSPEC CORPORATION
7018 Owensmouth Ave. Suite 103
Canoga Park, CA, 91303
Phone: 818-710-1281
Fax: 818-936-0121

PI E21-182
Revision C ;03/02/2011