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

Filaria

REF

Catalog No.E21-385

FOR RESEARCH Use Only

Intended Use

Immunospec Filarisis For the qualitative determination of serum antibodies, specifically IgG subclass 4, to filiarial antigens using the ELISA technique.

Summary

Filarisis is a disease caused by various filariases, which are vector borne human parasitic worms. These worms are nematodes that typically dwell in the lymphatic system or in the connective tissues.

Common species that can cause filarisis are *Wuchereria bancrofi, Brugia malayi, Brugia timori, Onchocerca volvulus, Mansonella perstans, Mansonella ozzardi,* and *Mansonella streptocerca.*

The species of infecting filaria will determine how the disease is expressed. Newly exposed individuals typically display acute symptomatology, with a rapid development to chronic and irreversible pathology.

Knowledge of the patient's travel history is imperative when diagnosing the disease. Blood smears and skin snips are primarily used to identify microfilariae while adult parasites may be found in the lymphatic system or tissue. This serum EIA detects IgG4 class antibodies to filarial antigens.

This disease can be found worldwide, although it is typically found in tropical regions. Lymphatic forms are found predominately in India, China, Indonesia, and South Pacific. Onchocerciasis (blindness) forms are found in Africa and Central America.

Principle of Procedure

During the first incubation, any antibodies in the patients' serum which are reactive with the antigen will bind to the coated wells. After washing to remove any unbound material, the Enzyme Conjugate is added. If antibodies have been bound to the wells, the Enzyme Conjugate will bind to these antibodies. After another series of washes, a Chromogen (tetramethylbenzidine or TMB) is added. If the Enzyme Conjugate is present, the Chromogen will change 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
Test Strips	Microwells containing filarial antigens - 96 test wells in a test strip holder.
Enzyme Conjugate	One (1) bottle containing 11 ml of anti-human IgG4 Peroxidase (HRP) in a stabilizing buffer with Thimerosal.
Positive Control	One (1) vial containing 1 ml of diluted Filarial -positive sera in buffer with Thimerosal.
Negative Control	One (1) vial containing 1 ml of diluted Filarial -negative sera in buffer with Thimerosal.
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 with Thimerosal.
Dilution Buffer	Two (2) bottles containing 30 ml of buffered protein solution with Thimerosal.
Stop Solution	One (1) bottle containing 11 ml of 1 M phosphoric acid.

Warnings/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.
- Treat all reagents and samples as potentially infectious materials. Use care to prevent aerosols and decontaminate any spills of samples.
- Negative Control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods.
- Positive Control has not been tested for Hepatitis B surface antigen or for the antibody to HIV and should be treated as potentially infectious material.
- 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 Conditions

- Reagents, strips and bottled components should be stored at 2-8 °C.
- Squeeze bottle containing diluted wash buffer may be stored at room temperature.

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 (15-25 °C) 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 (20X) 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 strongy hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thimerosal or <0.1% sodium azide is strongly recommended.
- Do not heat inactivate serum.
- Avoid repeated freezing and thawing of samples.
- ***Test samples**: Make a 1:51 dilution of patients' sera using the dilution buffer (e.g. 10 µl sera and 500 µl dilution buffer).*

Procedure

Materials Provided

- Filarial Serology Microwell ELISA Kit
- Microplate strip holder

Materials Required But Not Provided

- Pipettes
- Squeeze bottle for washing strips (narrow tip is recommended)
- Graduated Cylinder
- Reagent Grade (DI) water
- Tubes for serum dilutions
- Timer

Suggested Equipment

- ELISA plate reader with 450 and 620-650 nm filters

Proper Temperature

- All incubations are at room temperature (15 to 25 °C)

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 (1:51) test samples to the remaining wells. Tap the sides of the plate to mix. **Note**: Negative and positive controls are supplied prediluted. Do not dilute further. Controls must be included each time the kit is run.
3. Incubate at room temperature (15 to 25 ºC) for **15 minutes**, then wash.***** After last wash, slap wells against a clean absorbent towel to remove excess moisture.
4. Add **2 drops** of Enzyme Conjugate to each well. Cover the plate and tap the sides to mix.
5. Incubate at room temperature (15 to 25 ºC) for **15 minutes**, then wash.***** After last wash, slap wells against a clean absorbent towel to remove excess moisture.
6. Add **2 drops** of the Chromogen to each well. Tap the sides of the plate to mix.
7. Incubate at room temperature (15 to 25 ºC) for **10 minutes**.
8. Add **2 drops** of the Stop Solution and tap the sides of the plate to mix.
9. Read results visually or with an ELISA plate reader at a dual wavelength of 450/620-650 nm. Zero reader on air.

**** 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.

Quality Control

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must be over 0.3 OD units and the negative control must be under 0.2 OD units. Should the values fall outside these ranges, the kit should not be used.

Interpretation of Results

Spectrophotometer:

Zero ELISA reader on air. Read all wells using a bichromatic reading with filters at 450 nm and 620-650 nm.

Positive - Absorbance reading greater or equal to 0.2 OD units indicates the sample contains filarial antibodies.

Negative - Absorbance reading less than 0.2 OD units indicates the sample does not contain detectable levels of filarial antibodies.

Visual:

Positive: Any sample well that is obviously more yellow that the negative control well.

Negative: Any sample well that is not obviously more yellow than the negative control well.

NOTE: The negative control, as well as some samples, may show some slight color. A sample well must be obviously darker than the negative control well to be considered a positive result.

Test Limitations

- Test results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.
- A negative result can occur from an antibody level lower than the detection limits of this assay and treated Filaria patients.

Troubleshooting

Problem: Negative control has substantial color 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.

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, C.A. 91303 USA
(818)710-1281

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