Paragonimus IgM ELISA Kit

Para-M-96

Intended Use

The *Paragonimus* ELISA test is a semi-quantitative enzyme immunoassay for the detection of antibodies to *Paragonimus*, in samples of human serum or plasma. This test is intended to be performed by trained medical technologists only.

Summary and Explanation

Paragonimus is a parasitic lung fluke (flat worm). Cases of illness from infection occur after a person eats raw or undercooked infected crab or crayfish. The illness is known as paragonimiasis. *Paragonimus* infection also can be very serious if the fluke travels to the central nervous system, where it can cause symptoms of meningitis.

Paragonimus westermani and several other species are found throughout eastern, southwestern, and southeast Asia; (including China, the Philippines, Japan, Vietnam, South Korea, Taiwan, and Thailand). *P. africanus* is found in Africa, and *P. mexicanus* in Central and South America. There are several species of *Paragonimus* in other parts of the world that can infect humans. *P. kellicotti* is found in the midwestern and southern United States living in crayfish. Some human cases of infection have been associated with eating raw crayfish on river raft trips in the Midwest. *Paragonimus* has caused illness after ingestion of raw freshwater crabs.

The infection is transmitted by eating infected crab or crawfish that is either, raw, partially cooked, pickled, or salted. The larval stages of the parasite are released when the crab or crawfish is digested. They then migrate within the body, most often ending up in the lungs. In 6-10 weeks the larvae mature into adult flukes.

Adult flukes living in the lung cause lung disease. After 2-15 days, the initial signs and symptoms may be diarrhea and abdominal pain. This may be followed several days later by fever, chest pain, and fatigue. The symptoms may also include a dry cough initially, which later often becomes productive with rusty-colored or blood-tinged sputum on exertion. The symptoms of paragonimiasis can be similar to those of tuberculosis.

Assay Principle

The microwells are coated with *Paragonimus* 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.

Reagents

Item	Description	Symbol
Test Strips	Microwells containing *Paragonimus* antigens - 96 test wells in a test strip holder.	MT PLATE
Enzyme Conjugate	One (1) bottle containing 11 ml of anti-human IgM (u chain specific) conjugated to peroxidase.	CONJ
Positive Control	One (1) vial containing 2 ml of a surrogate marker.	CONTROL +
Negative Control	One (1) vial containing 2 ml of diluted human sera.	CONTROL −
Chromogen	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).	SUBS TMB
Wash Concentrate (20X)	Two (2) bottles containing 25 ml of concentrated buffer and surfactant.	WASH BUF
Dilution Buffer	Two (2) bottles containing 30 ml of buffered protein solution.	SPECM DIL
Stop Solution	One (1) bottle containing 11 ml of 1 M phosphoric acid.	SOLN

Statement of Warnings

- **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.
- 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. Positive 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)

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.

Specimen Collection And Handling

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.

Prepare a 1:100 dilution of serum or plasma (e.g. 5 ul serum + 500 ul Dilution Buffer).

Procedure

Materials Provided

Paragonimus IgG 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)

Proper Temperature

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

Test 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 (three for controls plus number of samples) and place in strip holder.
2. Dilute patient sera 1:100 using the Dilution Buffer (e.g. 5 µl sera and 500 µl dilution buffer).
3. Add **100 µl** of the negative control to well #1 and #2, **100 µl** of the positive control to well #3 and **100 µl** of the diluted test samples to the remaining wells.
4. Incubate at room temperature for **30 minutes**, then wash.* After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer if using manual washing.
5. Add **100 µl** of Enzyme Conjugate to each well.
6. Incubate at room temperature for **10 minutes**, then wash.* After last wash step, slap the wells on a clean absorbent towel to remove excess wash buffer if using manual washing.
7. Add **100 µl** of the Chromogen to each well.
8. Incubate at room temperature for **10 minutes**.
9. 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**.
10. Read within one hour of adding Stop Solution.

*** Washings consist of vigorously filling each well to overflowing and decanting contents three (3) separate times. If using automated washers; add 1 minute dwell time between washings and increase number of washes from three to five.**

Reading 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.

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.

Interpretation of the Test – ELISA Reader

1 – Calculate the average extinction value by taking the average OD value of the Negative Control.
2 – Add 0.200 to this average extinction value. This value is the cut-off value used in the Sample Index Calculation.

Example:
Negative Control 1 OD = 0.084
Negative Control 2 OD = 0.100
Average is 0.084 + 0.100 = 0.184 / 2 = 0.092 = Average Extinction Value

Cut-off value is the Average Extinction Value + 0.200 (in this example 0.092 + 0.200 = 0.292

3 – Determine the Sample Index by diving the patients OD value by the Cut-off value.

Example:
Patient OD value of 1.225
Cut-off value of 0.292

1.225 / 0.292 = 4.19

4 – Evaluate the Sample Index.
Negative = less than 1.0 Sample Index
Equivocal = 1.0 to 1.5
Positive = greater than 1.5

Limitations of The Procedure

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

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.

References

1. CDC, Atlanta, GA USA