DRG

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DRG® Schistosoma IgG (EIA-3512)

Revised 20 June 2011 rm (Vers. 3.1)

IVD

DIRECTIONS FOR USE
For *In Vitro* Diagnostic Use

INTENDED USE
For the qualitative determination of serum antibodies in humans, primarily IgG, to *Schistosoma* spp. using the ELISA technique.

PRINCIPLE OF PROCEDURE
During the first incubation, the antibodies in the patients' serum bind to the antigens in the test well. The next incubation allows the enzyme complex to bind to the antigen-antibody complex. After a few washings to remove unbound enzymes, a substrate is added that develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction, turning the blue assay color to yellow.

REAGENTS
- Microwell test strips containing *Schistosoma* SEA antigens - 96 test wells in a test strip holder.
- Enzyme conjugate: One (1) bottle containing 11 ml of Protein A Peroxidase (HRP) in a stabilizing buffer with Thimerosal.
- Positive control: One (1) vial containing 1 ml of diluted rabbit *Schistosoma*-positive sera in buffer with Thimerosal.
- Negative control: One (1) vial containing 1 ml of diluted *Schistosoma*-negative human sera in buffer with Thimerosal.
- TMB Substrate: One (1) bottle containing 11 ml of the (TMB).
- Wash concentrate solution (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 0.73 M phosphoric acid.

PRECAUTIONS
1. Do not use solutions if they precipitate or become cloudy.
2. Wash concentrate may show crystallization upon storage at 4° C. Crystallization will disappear after diluting to working strength.
3. 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.
4. Do not add azides to the samples or any of the reagents.
5. Controls and some reagents contain Thimerosal as a preservative.
6. Treat all sera as if capable of being infectious.

7. The negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. Since no test can offer complete assurance that infectious agents are not present, this product should be used under appropriate safety conditions that would be used for any potentially infectious agent.

STORAGE CONDITIONS

Reagents, strips and bottled components:

- Store between 2 - 8° C.
- Squeeze bottle containing diluted wash buffer may be stored at room temperature.

PREPARATION

Wash Buffer – Remove cap and add contents of bottle to 475 ml DI water. Place diluted wash buffer into a squeeze bottle.

Note: Washings consist of filling to the top of each well, shaking out the contents and refilling. Avoid generating bubbles in the wells during the washing steps.

Test samples – Make a 1:40 dilution of patients' sera using the dilution buffer.

Collection and Preparation of Serum

Serological specimens should be collected under aseptic conditions. Coagulate blood and remove serum.
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.
Avoid repeated freezing and thawing of samples.

MATERIALS

Materials Provided

Schistosoma Serology Microwell ELISA Kit

Materials Required But Not Provided

1. Pipettes
2. Squeeze bottle for washing strips
3. DI water
4. ELISA plate reader with a 450/620-650 nm filter (optionally, results can be read visually)
5. Tubes for serum dilutions

PROCEDURE

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Add 100 µl of negative control to well #1, 100 µl of positive control to well #2, and 100 µl of the diluted (1:40) test samples to the remaining wells.
   Note: Negative and positive controls are supplied as prediluted. Do not dilute.
3. Incubate at room temperature (15° to 25° C) for 10 minutes.
4. Shake out contents and wash 3 times with diluted wash buffer.*
5. Add 2 drops of enzyme conjugate to each well.
6. Incubate at room temperature for 10 minutes.
7. Shake out contents and wash 3 times with wash buffer.
8. Add 2 drops of Chromogen to every well.
9. Incubate at room temperature for 5 minutes.
10. Add 2 drops of stop solution.
11. Zero ELISA reader on air, read wells at 450 nm with a reference filter at 620-650 nm or read results visually.

* **Washings consist of using the diluted wash buffer to fill to the top of each well, shaking out the contents and refilling the wells for a total of 3 times.**

- Avoid generating bubbles in the wells during the washing steps.
- Controls must be included each time the kit is run.

TEST LIMITATIONS

Serological results should be used as an aid in diagnosis and should not be interpreted as diagnostic by themselves.

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.

Negative - Absorbance reading less than 0.2 OD units.

Visual: A sample should be interpreted as positive if the degree of color development is obvious and significant.

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 0.5 OD units and the negative control must be under 0.2 units. Should the values fall outside these ranges, the kit should not be used.

TROUBLESHOOTING

Problem: Negative control has substantial color development.
***Correction*:** Inadequate washings. Rerun test with more vigorous washings.

Performance Data

EIA-3512

	REFERENCE METHOD*	
	+	-
+	12	6
-	0	34

Positive Agreement: 100% (12/12)
Negative Agreement: 85% (34/40)

*Reference Method refers to a commercially available ELISA.

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