Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ Schistosoma IgG ELISA Kit

Cat # 8209-35

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

Test	Schistosoma IgG ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Qualitative : Positive, Negative
Sample	5 µL serum /plasma
Total Time	~ 25 min.
Shelf Life	12 Months from the manufacturing date
Specificity	100%
Sensitivity	100%

INTENDED USE

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

SUMMARY AND EXPLANATION

Schistosomiasis is a disease caused by parasitic worms of the genus Schistosoma. People are at risk for Schistosomiasis infection when they are exposed to the parasites, normally by bathing or swimming in contaminated water. Infection is transmitted when larvae from the parasite (usually freshwater snails) penetrates the skin and travels through the bloodstream. Acute and chronic symptoms result when the egg migration of the worms affects vital tissue and organs. Some affected people may be asymptomatic, but generally symptoms range skin irritation, to fever, intestinal and urinary tract infections, to possibly life-threatening complications. The disease has affected more than 200 million people worldwide, and has been classified as the second most common tropical disease following Malaria.

TEST PRINCIPLE

The principle of the Schistosoma ELISA test is a three-incubation process whereby the first incubation involves the coating of the wells with Schistosoma SEA antigens. 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 a 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. Coagulated blood and removed serum. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored 2-8ºC 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. Lipemic and strongly hemolytic serum should be avoided. Do not heat inactivate serum. Avoid repeated freezing and thawing of samples.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Plate:** Microwells containing *Schistosoma* SEA antigens - 96 test wells in a test strip holder.
2. **Enzyme Conjugate**: One (1) bottle containing 11 ml of Protein A Peroxidase (HRP) in a stabilizing buffer with Thimerosal.
3. **Positive Control**: One (1) vial containing 1 ml of diluted rabbit *Schistosoma*-positive sera in buffer with Thimerosal.
4. **Negative Control:** One (1) vial containing 1 ml of diluted *Schistosoma*-negative human sera in buffer with Thimerosal.
5. **TMB Substrate Solution:** One (1) bottle containing 11 ml of the TMB tetramethylbenzidine (TMB).
6. **Wash Concentrate 20X**: One (1) bottle containing 25 ml of concentrated buffer and surfactant with Thimerosal.
7. **Dilution Buffer:** Two (2) bottles containing 30 ml of buffered protein solution with Thimerosal
8. **Stop Solution**: One (1) bottle containing 11 ml of 0.73 M phosphoric acid.

Materials required but not provided

1. Pipettes
2. Squeeze bottle for washing strips
3. DI water
4. Tubes for serum dilutions
5. ELISA plate reader with a 450 nm and a 620-650 nm filter (optional if results are read visually.

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.

ASSAY 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 °C to 25 °C) for 10 minutes.
4. Shake out contents and wash 3 times with diluted wash buffer.*
5. Add 100 µl 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 100 µl of Chromogen to every well.
9. Incubate at room temperature for 5 minutes.
10. Add 100 µl 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.

Diagnostic Automation/Cortez Diagnostics, Inc.
23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302 USA Phone: 818.591.3030 Fax 818.591.8383
Email:onestep@rapidtest.com Website: www.rapidtest.com

Avoid generating bubbles in the wells during the washing steps.

Controls must be included each time the kit is run.

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.

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

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.

PERFORMANCE CHARACTERISTICS

		Reference Method *	
		+	-
Diagnostic Automation,Inc.	+	12	0
	-	0	65

Positive Agreement: 100% (12/12)
Negative Agreement: 100% (65/65)

*Reference Method refers to a commercially available ELISA.

LIMITATIONS OF PROCEDURE

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

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

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

REFERENCES

1. Carvalho EM, Lima AAM. Schistosomiasis (Bilharziasis). In: Goldman L, Schafer AI, eds. Cecil Medicine. 24th ed. Philadelphia, Pa: Saunders Elsevier; 2011:chap 363.
2. Maguire JH. Trematodes (schistosomes and other flukes). In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases. 7th ed. Philadelphia, Pa: Elsevier Churchill Livingstone; 2009:chap 289.

ISO 13485
ISO 9001

Diagnostic Automation/Cortez Diagnostics, Inc.
23961 Craftsman Road, Suite E/F,
Calabasas, California 91302 USA

Date Adopted	Cat # 8209-35
2013-10-01	AccuDiag™- Schistosoma IgG ELISA -2013
EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu

Revision C Date: 12-12-2013