

# **Schistosoma mansoni IgG ELISA**

Enzyme immunoassay for the diagnosis of human schistosomiasis

96 assays on individual wells for in vitro diagnostic use and for professional laboratory use



Instructions for use for article N° **9600**  
EC reg. H-CH/CA01/IVD/17983- UDI-DI: 07640158219607



## **Intended use:**

The Bordier *Schistosoma mansoni* IgG ELISA kit is intended for the quantitative detection of IgG antibodies against *Schistosoma mansoni* and *Schistosoma haematobium* in human serum. Serology is an aid for diagnosis and cannot be used as the sole method of diagnosis.

## **Background:**

Schistosomiasis, also known as bilharzia, is caused by parasitic trematode worms such as *Schistosoma mansoni*, *S. haematobium*, or *S. japonicum*. Humans can be infected by contacting contaminated water with Schistosoma cercariae which can percutaneously enter the body through exposed skin. During a period of several weeks, the juvenile parasites migrate through host tissue and subsequently develop into adult worms inside the blood vessels of the body. Once mature, the worms mate and females produce eggs. Some of these eggs travel to the bladder or intestine and are passed into the urine or stool. Symptoms are mainly caused by the body's reaction to parasite eggs present in the affected tissues. Main symptoms are ash or itchy skin within few days; fever, chills, cough, and muscle aches within 1-2 months; abdominal pain, enlarged liver and blood in stool or urine during chronic stage. Diagnosis is based on egg detection in stool or urine, and a positive result by serological testing.

## **Principle and presentation:**

The kit provides all the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on breakable microtitration wells sensitized with ***Schistosoma mansoni*** soluble antigens. Specific antibodies in the sample will bind to these antigens and washing will remove unspecific antibodies. The presence of parasite specific antibodies is detected with a Protein A - alkaline phosphatase conjugate. A second washing step will remove unbound conjugate. Revealing bound antibodies is made by the addition of pNPP substrate which turns yellow in the presence of alkaline phosphatase. Color intensity is proportional to the amount of *Schistosoma mansoni* specific antibodies in the sample. Potassium phosphate is added to stop the reaction. Absorbance at 405 nm is read using an ELISA microplate reader.

The test can be performed with automatic systems, but this must be validated by the user.

## **Material contained in the kit (96 assays):**

<b>WELL</b>					
	9600-01	Breakable ELISA strips sensitized with <b><i>Schistosoma mansoni</i></b> soluble antigens		96	wells
DILB	9600-02	Dilution buffer (10 x) concentrate, coloured purple		50	ml
WASH	9600-03	Washing solution (10 x) concentrate		50	ml
ENZB	9600-04	Enzyme buffer		50	ml
STOP	9600-05	Stopping solution (0.5M K <sub>3</sub> PO <sub>4</sub> )		25	ml
CONTROL -	9600-06	Negative control serum (20 x), green cap		200	µl
CONTROL -/+	9600-07	Weak positive control serum (cut off, 20 x), yellow cap		200	µl
CONTROL +	9600-08	Positive control serum (20 x), red cap		200	µl
CONJ	9600-09	Protein A - alkaline phosphatase conjugate (50 x), purple cap		300	µl
SUBS	9600-10	Phosphatase substrate (para-nitrophenylphosphate) Multipipette reservoir, 25 ml Frame for ELISA 8-well holder		20	tablets piece piece

## **Shelf life and storage:**

Store the kit at 2° to 8°C (transport at ambient temperature), avoid long term exposure of the components to direct light. The expiry date and the lot number of the kit are printed on the side of the box. After initial opening, all reagents are stable until the expiry date when stored at 2-8°C.

## **Equipment needed but not provided with the kit:**

Pipettes (ml and  $\mu$ l). Flasks. Dilution tubes. Adhesive tape to cover wells during incubations. Distilled water. Incubator set at 37°C. ELISA reader set at 405 nm. Manual or automatic equipment for rinsing wells. Vortex mixer. Timer.

## **Preparation of reagents before use:**

Bring all reagents to room temperature and mix before use.

**ELISA wells:** open side of aluminum bag 9600-01 and remove number of wells needed (one for blank, three for controls plus the number of samples). Place sensitized wells in 8-well holder(s). If needed, complete the empty positions in the holder with used wells. Insert holder(s) in the frame in the correct orientation. Reseal open package with desiccant pad.

**Dilution buffer:** dilute dilution buffer (10 x) concentrate 9600-02, 1/10 in distilled water. This is used for the dilution of controls, samples and conjugate. The diluted buffer is stable for 2 months at 2-8°C.

**Washing solution:** dilute washing solution (10 x) concentrate 9600-03, 1/10 in distilled water. You may also use your own washing solution. Avoid buffers containing phosphate which could inhibit the enzymatic activity of the alkaline phosphatase. The diluted washing solution is stable for 2 months at 2-8°C.

**Control sera:** dilute 10  $\mu$ l control sera 9600-06 to -08 in 190  $\mu$ l dilution buffer solution (final dilution 1/20). The diluted control sera are stable for 2 months at 2-8°C.

**Conjugate:** dilute conjugate 9600-09 in dilution buffer solution (final dilution 1/50). Dilute conjugate on the day of the assay. Do not store diluted conjugate.

**Substrate solution:** dissolve tablet(s) of phosphatase substrate 9600-10 in undiluted enzyme buffer 9600-04 (1 tablet in 2.5 ml buffer). Vortex until complete dissolution of the tablet(s). Dilute substrate on the day of the assay and protect the tube from direct light. Tablets and substrate solutions should be colourless or should have only a slight yellow tinge. If a tablet or a substrate solution turns yellow, it may have been partially hydrolysed and should be discarded. Do not store the substrate solution.

**Stopping solution:** use reagent 9600-05 undiluted.

## **Specimen collection and preparation:**

Use human serum. Serum should be stored at 2-8°C if analysed within a few days, otherwise store at -20°C or lower. Avoid repeated freezing and thawing.

Vortex samples and dilute 1/201 in dilution buffer solution (for instance 5  $\mu$ l sample in 1.0 ml).

## **Warnings and precautions:**

Toxic compounds are found in following concentration:

Component	Reference	Sodium azide ( $N_aN_3$ )	Merthiolate
Dilution buffer (10 x)	9600-02	0.1 %	0.02 %
Washing solution (10 x)	9600-03	0.05 %	/
Enzyme buffer	9600-04	0.01 %	/
Control sera (20 x)	9600-06 to -08	0.1 %	0.02 %
Conjugate (50 x)	9600-09	0.1 %	/

At the used concentrations, sodium azide and merthiolate do not have any toxicological risk on contact with skin and mucous membranes.

- The stopping solution 9600-05 (0.5 M  $K_3PO_4$ ) is irritant.
- The negative, weak positive, and positive control sera (9600-06 to -08) are from rabbits.
- Treat all reagents and samples as potentially infectious material.
- Do not interchange reagents of different lots or Bordier ELISA kits.
- Do not use reagents from other manufacturers with reagents of this kit.
- Do not use reagents after their expiry date.
- Close reagent vials tightly immediately after use and do not interchange screw caps to avoid contamination.
- Use separate and clean pipettes tips for each sample.
- Do not re-use microwells.
- Avoid deterioration of the microwells by mechanical action (tips/cones, nozzles).
- The descriptions of symbols used on the labels can be found on the website [www.bordier.ch](http://www.bordier.ch).

## **Disposal consideration:**

All materials used for this test are generally considered as hazardous waste. Refer to national and regional laws and regulations for the disposal of hazardous waste.

## **Procedure:**

When running the assay, avoid the formation of bubbles in the wells.

### **Step 1: Blocking:**

Fill completely wells with dilution buffer solution.

Incubate for 5 to 15 minutes at ambient temperature (blocking).

Remove dilution buffer by aspiration or by shaking the wells over the sink.

### **Step 2: Incubation with samples:**

Fill the first well of the first strip with 100 µl dilution buffer only (no-serum blank).

Fill the subsequent three wells with respectively 100 µl diluted negative, weak positive (cut off) and positive control serum. For assays of more than 25 samples, we recommend to fill the three last wells with control sera as a duplicate.

Fill remaining wells with the diluted samples (100 µl each).

Cover wells with adhesive tape and incubate for 30 minutes at 37°C.

Remove sera and wash 4 x with ~ 250 µl washing solution.

### **Step 3: Incubation with conjugate:**

Distribute 100 µl diluted conjugate in each well (including no-serum blank).

Cover wells with adhesive tape and incubate for 30 minutes at 37°C.

Remove conjugate and wash 4 x with ~ 250 µl washing solution.

### **Step 4: Incubation with substrate:**

Distribute 100 µl substrate solution per well.

Cover wells with adhesive tape and incubate for 30 minutes at 37°C.

Stop the reaction by the addition of 100 µl stopping solution to each well.

### **Step 5: Measurement of absorbances:**

If needed, wipe the bottom of the wells and eliminate bubbles. Measure absorbances at 405 nm within 1 hour after the addition of stopping solution.

## **Interpretation:**

Subtract the value of the no-serum blank from all measured values. When applicable calculate the mean absorbance values of duplicated serum controls. The test is valid if the following criteria are met:

- Absorbance (A) of positive control > 1.200
- A of weak positive control > 10 % of A of positive control
- A of negative control < 8 % of A of positive control
- A of no-serum blank < 0.350

Quality controls of current lots are published on our website: [www.bordier.ch](http://www.bordier.ch).

The antibody concentration of the weak positive (cut off) serum 9600-07 has been set to discriminate optimally between sera of clinically documented cases of schistosomiasis and healthy human sera.

The cut off index of a sample is defined, after subtraction of the no-serum blank, as:

$$\text{Index} = \frac{\text{Absorbance sample}}{\text{Absorbance cut off serum}}$$

The result is **negative** when the index of the analyzed sample is lower than 1.0. In this case, the IgG antibody concentration against ***Schistosoma mansoni*** antigens is clinically non-significant.

The result is **positive** when the index of the analyzed sample is higher than 1.0. In this case, the IgG antibody concentration against ***Schistosoma mansoni*** antigens is considered as clinically significant. It indicates that the patient has had a contact with the parasite.

A grey zone could be defined by each laboratory according to its patients population. In case of borderline or doubtful results, we recommend repeating the test again 2-4 weeks later with a fresh sample.

In case of positive or doubtful result, we recommend performing a confirmation test (most often by western blot) if such a test is available or required by national regulations.

## **Analytical performances:**

### **Analytical specificity:**

A specificity of 87% was found with 178 sera of patients with other parasitic infections. Cross-reactivity mainly occur in patients with filarioses and leishmaniosis.

No positive or negative interference was observed with supraphysiological concentrations of hemoglobin, lipids or bilirubin in sera supplemented with interferents.

### **Precision:**

Repeatability were assessed by testing 2 human serum samples in 24 wells on 1 assay.

Reproducibility were assessed by testing the 2 human serum samples on 10 different assays.

	Repeatability		Reproducibility	
	Sample 1	Sample 2	Sample 1	Sample 2
<b>Average (absorbance)</b>	0.412	1.249	0.407	1.246
<b>Standard deviation (absorbance)</b>	0.031	0.067	0.029	0.076
<b>Variation coefficient (%)</b>	7.6	5.3	7.1	6.1

The following performances cannot be evaluated because there is no certified reference material for this analysis:

- Analytical sensitivity (limits of detection and quantitation)
- Accuracy
- Trueness
- Measuring range
- Linearity

## **Clinical performances:**

### **Diagnostic sensitivity:**

A sensitivity of 93% was found with 99 sera from patients with parasitologically proven schistosomiasis (36/40 *S. mansoni* and 28/30 *S. haematobium*) or with positive specific serology on western-blot (28/29).

### **Diagnostic specificity:**

A specificity of 99% was found with 122 sera of blood donors (Swiss).

### **Positive and negative predictive value:**

A PPV of 88% and a NPV of 85% were found with the populations mentioned above.

### **Expected values in normal and affected populations:**

In a normal population of 180 Swiss blood donors and 96 sera from a Swiss infectiology unit, the expected Index value is 0.20. In an affected population of 31 sera from patients suffering from schistosomiasis, the expected Index value is 3.92.

### **Incidents :**

Any serious incident occurring in connection with the device shall be notified to the manufacturer and to the competent authority of the Member State in which the user and/or the patient is established.

### **Limitations:**

Diagnosis of an infectious disease should not be established on the basis of a single test results. A precise diagnosis should take into consideration endemic situation, clinical history, symptomatology, imaging as well as serological data.

In immunocompromised patients and newborns, serological data are of limited value.

### **References:**

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