Ascaris IgG ELISA

Enzyme immunoassay for the diagnosis of human ascariasis

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

BORDIER AFFINITY PRODUCTS

Instructions for use for article N° **9250**
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CE

Intended use:

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

Background:

Ascariasis is a worldwide helminthic infection caused by some soil transmitted helminths of the genus *Ascaris* (mostly *A. Lumbricoides* and *A. suum*). It occurs most often in children in tropical and subtropical regions, especially in areas with poor sanitation and hygiene. Humans can be infected by accidentally ingesting infectious eggs upon contaminated soil, water or food. After ingestion of eggs, larvae hatch in the small intestine and migrate mainly through the bloodstream into the liver, and then to the lungs, where both they can cause damages, which are dependent upon infection intensity. From the lungs, moulded larvae travel via trachea to the throat, where most of them are swallowed, and thus reach the gastro-intestinal tract. The larvae mature into adult worms in the small intestine, where they live and sexually reproduce for a very long time period, up to years. Most infected people do not show any symptoms. However, in some cases, symptoms appear on the lung stage (persistent cough, shortness of breath and wheezing) or at the intestinal stage (abdominal pain, nausea, vomiting and diarrhoea). Diagnosis is based on the detection of eggs in stool, signs and symptoms plus a history of exposure 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 ***Ascaris*** 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 *Ascaris* 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):

WELL	9250-01	Breakable ELISA strips sensitized with ***Ascaris*** soluble antigens	96	wells
DILB	9250-02	Dilution buffer (10 x) concentrate, coloured purple	50	ml
WASH	9250-03	Washing solution (10 x) concentrate	50	ml
ENZB	9250-04	Enzyme buffer	50	ml
STOP	9250-05	Stopping solution (0.5M K_3PO_4)	25	ml
CONTROL -	9250-06	Negative control serum (20 x), green cap	200	µl
CONTROL -/+	9250-07	Weak positive control serum (cut off, 20 x), yellow cap	200	µl
CONTROL +	9250-08	Positive control serum (20 x), red cap	200	µl
CONJ	9250-09	Protein A - alkaline phosphatase conjugate (50 x), purple cap	300	µl
SUBS	9250-10	Phosphatase substrate (para-nitrophenylphosphate)	20	tablets
		Multipipette reservoir, 25 ml	1	piece
		Frame for ELISA 8-well holder	1	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 µl). 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 9250-01 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 9250-02, 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 9250-03, 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 µl control sera 9250-06 to -08 in 190 µl dilution buffer solution (final dilution 1/20). The diluted control sera are stable for 2 months at 2-8°C.

Conjugate: dilute conjugate 9250-09 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 9250-10 in undiluted enzyme buffer 9250-04 (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 9250-05 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 µl 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)	9250-02	0.1 %	0.02 %
Washing solution (10 x)	9250-03	0.05 %	/
Enzyme buffer	9250-04	0.01 %	/
Control sera (20 x)	9250-06 to -08	0.1 %	0.02 %
Conjugate (50 x)	9250-09	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 9250-05 (0.5 M K_3PO_4) is irritant.
- The negative, weak positive, and positive control sera (9250-06 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.

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 > 13 % 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.

The antibody concentration of the weak positive (cut off) serum 9250-07 has been set to discriminate optimally between sera of clinically documented cases of ascariasis 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 ***Ascaris*** 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 ***Ascaris*** 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 66% was found with 41 sera of patients with other parasitic infections. Cross-reactivity mainly occur in patients with toxocarosis and trichinellosis.
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 differents assays.

	Repeatability		Reproducibility	
	Sample 1	**Sample 2**	**Sample 1**	**Sample 2**
Average (absorbance)	0.627	1.633	0.692	1.851
Standard deviation (absorbance)	0.038	0.068	0.034	0.067
Variation coefficient (%)	6.1	4.2	5.0	3.6

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 81% was found with 27 sera positive with *Ascaris suum* crude extract antigen and negative with *Toxocara canis* Bordier ELISA kit.

Diagnostic specificity:

A specificity of 75% was found with 44 sera positive with Ascaris *suum* crude extract antigen and positive with *Toxocara canis* Bordier ELISA kit. A specificity of 96% was found with 181 sera of blood donors (Swiss). A specificity of 98% was found with 96 sera from patients of an infectiology unit (Swiss).
A negative result was found for 147 of the 150 negative samples with another commercial technique detecting anti-*Ascaris* antibodies. A positive result was found for 8 of the 9 samples positive with this technique.

Positive and negative predictive value:

A PPV of 52% and a NPV of 92% were found with the populations mentioned above.

Expected values in normal and affected populations:

In a normal population of 180 Swiss blood donors, 96 sera from a Swiss infectiology unit and 44 sera positive with *Ascaris suum* crude extract antigen and positive with *Toxocara canis* Bordier ELISA kit, the expected Index value is 0.46. In an affected population of 27 sera positive with *Ascaris suum* crude extract antigen and negative with *Toxocara canis* Bordier ELISA kit, the expected Index value is 1.21.

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 estabished 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:

Schneider, R. and Auer, H. (2016) Incidence of Ascaris suum-specific antibodies in Austrian patients with suspected larva migrans visceralis (VLM) syndrome. Parasitology Research **115**, 1213-1219.
Dana D, Vlaminck J, Ayana M, Tadege B, Mekonnen Z, Geldhof P, et al. (2020) Evaluation of copromicroscopy and serology to measure the exposure to Ascaris infections across age groups and to assess the impact of 3 years of biannual mass drug administration in Jimma Town, Ethiopia. PLoS Negl Trop Dis **14**(4).

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