Entamoeba histolytica IgG ELISA

Enzyme immunoassay for the diagnosis of human invasive amebiasis

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

BORDIER AFFINITY PRODUCTS

Instructions for use for article N° **9550**
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Intended use:

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

Background:

Amebiasis is caused by the protozoan *Entamoeba histolytica*, a pathogenic amoeba. Humans can be infected by accidentally ingesting amoebic cysts in contaminated food or water. The most frequent symptoms appear during the intestinal stage (stomachache and diarrhea). However, in some cases, the parasite will become extra-intestinally invasive, and thus lead to abscess formation mainly in the liver. Patients will suffer mainly from fever and abdominal pain. Diagnosis of invasive amebiasis is based on imaging techniques, such as CT scans, ultrasonography, and MRIs to detect hepatic lesion and a positive result by serological testing. Serology is also used for excluding amoebiasis in the frame of differential diagnosis with other liver diseases.

Principle and presentation:

The kit provides all the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on breakable microtitration wells sensitized with ***Entamoeba histolytica*** soluble trophozoite 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 *Entamoeba histolytica* 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 is manual but can be performed with automatic systems, which must be validated by the user.

Material contained in the kit (96 assays):

WELL	9550-01	Breakable ELISA strips sensitized with ***Entamoeba histolytica*** soluble trophozoite antigens	96	wells
DILB	9550-02	Dilution buffer (10 x) concentrate, coloured purple	50	ml
WASH	9550-03	Washing solution (10 x) concentrate	50	ml
ENZB	9550-04	Enzyme buffer	50	ml
STOP	9550-05	Stopping solution (0.5M K_3PO_4)	25	ml
CONTROL -	9550-06	Negative control serum (20 x), green cap	200	µl
CONTROL -/+	9550-07	Weak positive control serum (cut off, 20 x), yellow cap	200	µl
CONTROL +	9550-08	Positive control serum (20 x), red cap	200	µl
CONJ	9550-09	Protein A - alkaline phosphatase conjugate (50 x), purple cap	300	µl
SUBS	9550-10	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 between +2°C and +8°C (transport validated between -20°C and +37°C for 21 days), 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 between +2°C and +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 aluminium bag 9550-01 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 9550-02, 1/10 in distilled water. This is used for the dilution of controls, samples, and conjugate. The diluted buffer is stable for 2 months between +2°C and +8°C.

Washing solution: dilute washing solution (10 x) concentrate 9550-03, 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 between +2°C and +8°C.

Control sera: dilute 10 µl control sera 9550-06 to -08 in 190 µl dilution buffer solution (final dilution 1/20). The diluted control sera are stable for 2 months between +2°C and +8°C.

Conjugate: dilute conjugate 9550-09 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 9550-10 in undiluted enzyme buffer 9550-04 (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 9550-05 undiluted.

Specimen collection and preparation:

Use human serum. Store between +2°C and +8°C if analyzed within 7 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). Do not store diluted samples.

Warnings and precautions:

Toxic compounds are found in following concentration:

Component	Reference	Sodium azide (N_aN_3)	Merthiolate
Dilution buffer (10 x)	9550-02	0.1 %	0.02 %
Washing solution (10 x)	9550-03	0.05 %	/
Enzyme buffer	9550-04	0.01 %	/
Control sera (20 x)	9550-06 to -08	0.1 %	0.02 %
Conjugate (50 x)	9550-09	0.1 %	/

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

Component	Dangerous component	Danger pictogram	Hazard statement	Precautionary statement
Stopping solution	Potassium phosphate, tribasic		Causes serious eye damage	Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

- The negative, cut off and positive control sera (9550-06 to -08) are of animal origin (rabbits) and must be handled with care.
- 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: Preincubation:
Fill wells with 250 µl of dilution buffer solution.
Incubate for 5 to 15 minutes at ambient temperature.
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 filling 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 > 17 % of A of positive control
- A of negative control < 12 % of A of positive control
- A of no-serum blank < 0.350

In case of sample providing signal overflowing the microplate reader measurement range, the value corresponding to the upper measurement range of the reader should be attributed.

Quality controls of current lots are published on our website: www.bordier.ch.

The antibody concentration of the weak positive (cut off) serum 9550-07 has been set to discriminate optimally between sera of clinically documented cases of invasive amebiasis 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 ***Entamoeba histolytica*** antigens is clinically non-significant.
The result is **positive** when the index of the analyzed sample is higher or equal to **1.0**. In this case, the IgG antibody concentration against ***Entamoeba histolytica*** 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 88.5% was found with 139 sera of patients with other parasitic infections. Cross-reactivity mainly occur in patients with cryptosporidiosis, giardiasis, filarioses and cystic echinococcosis.
No positive or negative interference was observed with supraphysiological concentrations of hemoglobin, lipids or bilirubin in sera supplemented with interferents.

Precision:

Repeatability was assessed by testing 2 human serum samples in 24 wells on 1 assay.
Reproducibility was assessed by testing the 2 human serum samples in duplicate on 10 different assays.

	Repeatability		Reproducibility	
	Sample 1	**Sample 2**	**Sample 1**	**Sample 2**
Average (absorbance)	0.612	2.394	0.649	2.449
Standard deviation (absorbance)	0.040	0.162	0.041	0.166
Variation coefficient (%)	6.5	6.8	6.3	6.8

Clinical performances:

Diagnostic sensitivity:

A sensitivity of 100% was found with 83 sera of patients suffering from invasive amebiasis.
A sensitivity of 71.4% was found with 14 sera of patients suffering from intestinal amebiasis.

Diagnostic specificity:

A specificity of 95.3% was found with 80 sera of blood donors (France), 18 sera of stool donors for fecal transplantation, 60 sera of dysimmune patients and 33 sera of patients suffering from other hepatic diseases.

Positive and negative predictive value:

A PPV of 90.3% and a NPV of 100% were found with the populations mentioned above (intestinal amebiasis excluded).

Expected values in normal and affected populations:

An expected Index value of 0.35 was found in the normal population and 4.19 in the affected population (intestinal amebiasis excluded).

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 result. 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:

Nicholls, R.S., I Restrepo, M., Duque, S., Consuelo Lopez, M., Corredor, A. (1994) Standardization and evaluation of Elisa for the serodiagnostic of amoebic liver abscess. Mem Inst Oswaldo Cruz, Rio de Janeiro. 89: 53-58.
Visser, L.G., Verweij, J.J., Van Esbroeck, M., Edeling, W.M., Clerinx, J. Polderman A.M. (2006) Diagnostic methods for differentiation of E. histolytica and E. dispar in carriers : performance and clinical implications in a non-endemic setting. Int. journal of med. microbiol. 296 : 397-403.

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