Echinococcus multilocularis (Em18) IgG ELISA

Enzyme immunoassay for the serological follow-up of human alveolar echinococcosis

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

BORDIER AFFINITY PRODUCTS

Instructions for use for article N° **9310**
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Intended use:

The Bordier *Echinococcus multilocularis* (Em18) IgG ELISA kit is intended for the qualitative detection of IgG antibodies against Em18 antigen of *Echinococcus multilocularis* in human serum. This test is intended for post-operative and/or post-therapeutic follow-up of infected patients.

Background:

Alveolar echinococcosis is caused by the larval stage of *Echinococcus multilocularis*, a tapeworm found in foxes, coyotes, dogs and some other canids. Humans can be infected by accidentally ingesting tapeworm eggs upon contaminated food or water. The larval forms of *E. multilocularis* do not fully mature into fertil cysts in humans, but continuous proliferation of vesicles that invade and destroy surrounding tissues will cause, in a tumor-like manner, liver disfunction. The parasite can spread into other organs such as the lungs and brain. The main symptoms are abdominal pain, asthenia, hepatomegaly and jaundice. Diagnosis is based on imaging techniques such as CT scans to visually detect parasitic masses and respective diffuse cyst-like structures. Serologic tests are used for screening populations at risk, and for follow-up AE patients after treatment.

Principle and presentation:

The kit provides all the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on breakable microtitration wells sensitized with ***Echinococcus multilocularis*** Em18 recombinant antigen. 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 *Echinococcus multilocularis* 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	9310-01	Breakable ELISA strips sensitized with ***Echinococcus multilocularis*** Em18 recombinant antigen	96	wells
DILB	9310-02	Dilution buffer (10 x) concentrate, coloured purple	50	ml
WASH	9310-03	Washing solution (10 x) concentrate	50	ml
ENZB	9310-04	Enzyme buffer	50	ml
STOP	9310-05	Stopping solution (0.5M K_3PO_4)	25	ml
CONTROL -	9310-06	Negative control serum (20 x), green cap	200	µl
CONTROL -/+	9310-07	Weak positive control serum (cut off, 20 x), yellow cap	200	µl
CONTROL +	9310-08	Positive control serum (20 x), red cap	200	µl
CONJ	9310-09	Protein A - alkaline phosphatase conjugate (50 x), purple cap	300	µl
SUBS	9310-10	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 9310-01 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 9310-02, 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 9310-03, 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 9310-06 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 9310-09 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 9310-10 in undiluted enzyme buffer 9310-04 (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 9310-05 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)	9310-02	0.1 %	0.02 %
Washing solution (10 x)	9310-03	0.05 %	/
Enzyme buffer	9310-04	0.01 %	/
Control sera (20 x)	9310-06 to -08	0.1 %	0.02 %
Conjugate (50 x)	9310-09	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 (9310-06 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 > 3 % of A of positive control
- A of negative control < 3 % 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 9310-07 has been set to discriminate optimally between sera of clinically documented cases of alveolar echinococcosis 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 ***Echinococcus multilocularis*** 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 ***Echinococcus multilocularis*** antigens is considered as clinically significant.
Decrease or negativation of anti-recEm18 serum antibody levels indicates a complete surgical resection of the parasite lesion or an inactivation of the parasite by drug treatment.
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 100% was found with 12 sera of patients with other parasitic infections (6 cystic echinococcosis, 3 undifferentated echinococcosis and 3 alternative diagnoses).
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)	0.186	1.260	0.157	1.123
Standard deviation (absorbance)	0.022	0.072	0.016	0.076
Variation coefficient (%)	11.8	5.7	10.5	6.8

Clinical performances:

Diagnostic sensitivity:

Paired pre- and post-surgical serum samples of 12 patients with confirmed alveolar echinococcosis and having had a radical or non-radical surgery were studied. Pre-surgically, 9 patients (75%) had an index >1. Among these patients, 5 had negative post-surgical results. But in all 12 patients, post-surgical Em18 antibody levels dropped and were significantly lower than in pre-surgical samples.
Serum samples of 25 patients with confirmed alveolar echinococcosis without surgery but with stable disease under antiparasitic chemotherapy were studied. 18 (72%) of them had an index >1 (median index 6.3). Serum samples of 7 patients with confirmed alveolar echinococcosis without surgery but with progressive disease under antiparasitic chemotherapy were studied. 6 (86%) of them had an index >1 (median index 13.8).

Diagnostic specificity:

A specificity of 98 % was found with 99 sera of blood donors (Swiss). A specificity of 94% was found with 100 sera from patients of an infectiology unit (Swiss).

Positive and negative predictive value:

A PPV of 80% and a NPV of 96% were found with the populations mentioned above.

Expected values in normal and affected populations:

In a normal population of 99 Swiss blood donors and 100 sera from a Swiss infectiology unit, the expected Index value is 0.33. Data on the expected Index value in an affected population was not available.

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:

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