

Leishmania infantum IgG ELISA

Enzyme immunoassay for the diagnosis of human leishmaniasis

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



Instructions for use for article N° 9500

UDI-DI: 07640158219508



Intended use:

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

Background:

Leishmaniasis is a vector-borne disease transmitted by sandflies. Infection occurs by different species of the obligate intracellular protozoa of the genus *Leishmania*. Human infection is caused by about 21 of 30 species known to infect mammals. The most common disease forms are cutaneous leishmaniasis, which causes skin disorders, and visceral leishmaniasis, which affects usually spleen, liver, and bone marrow. The main symptoms are fever, enlarged spleen, and cutaneous affections. Diagnosis of visceral leishmaniasis is based on a positive result by serological testing, and positive PCR on bone marrow aspirates or skin-biopsies.

Principle and presentation:

The kit provides all the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on breakable microtitration wells sensitized with *Leishmania infantum* somatic promastigote 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 *Leishmania infantum* 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	9500-01	Breakable ELISA strips sensitized with <i>Leishmania infantum</i> somatic promastigote antigens	96	wells
DILB	9500-02	Dilution buffer (10 x) concentrate, coloured purple	50	ml
WASH	9500-03	Washing solution (10 x) concentrate	50	ml
ENZB	9500-04	Enzyme buffer	50	ml
STOP	9500-05	Stopping solution (0.5M K ₃ PO ₄)	25	ml
CONTROL -	9500-06	Negative control serum (20 x), green cap	200	µl
CONTROL -/+	9500-07	Weak positive control serum (cut off, 20 x), yellow cap	200	µl
CONTROL +	9500-08	Positive control serum (20 x), red cap	200	µl
CONJ	9500-09	Protein A - alkaline phosphatase conjugate (50 x), purple cap	300	µl
SUBS	9500-10	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 between +20°C and +30°C for 1 hour minimum and mix before use.

ELISA wells: open side of aluminium bag 9500-01 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 9500-02, 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 9500-03, 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 9500-06 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 9500-09 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 9500-10 in undiluted enzyme buffer 9500-04 (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 9500-05 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 (NaN ₃)	Merthiolate
Dilution buffer (10 x)	9500-02	0.1 %	0.02 %
Washing solution (10 x)	9500-03	0.05 %	/
Enzyme buffer	9500-04	0.01 %	/
Control sera (20 x)	9500-06 to -08	0.1 %	0.02 %
Conjugate (50 x)	9500-09	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 (9500-06 to -08) are of animal origin (dogs) 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 between +20°C and +30°C.

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 > 10 % of A of positive control
- A of negative control < 8 % 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 9500-07 has been set to discriminate optimally between sera of clinically documented cases of leishmaniosis 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 *Leishmania infantum* 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 *Leishmania infantum* 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 100% was found with 15 sera of patients with other parasitic infections. Cross-reactivity might occur in some other parasitic infections such as african trypanosomiasis, Chagas disease and cutaneous and mucocutaneous leishmaniosis.

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)	1.005	1.766	1.381	2.187
Standard deviation (absorbance)	0.061	0.086	0.097	0.117
Variation coefficient (%)	6.1	4.9	7.0	5.3

Clinical performances:

Diagnostic sensitivity:

A sensitivity of 93% was found with 29 sera of immunocompetent patients (HIV-) suffering from visceral leishmaniosis due to *L. infantum*. A sensitivity of 67% was found with 21 sera HIV-*Leishmania* co-infected patients. A negative serology and a positive culture may occur in these patients when they are immunosuppressed or infected with other *Leishmania* species such as *L. major* or *L. braziliensis*.

Diagnostic specificity:

A specificity of 100% was found with 99 sera of blood donors (Swiss).

Positive and negative predictive value:

A PPV of 100% and a NPV of 98% were found with the HIV- population mentioned above and 100% and 93% with the HIV+ population mentioned above.

Expected values in normal and affected populations:

In a normal population of 99 Swiss blood donors, the expected Index value is 0.39. In an affected population of 13 sera from patients suffering from visceral leishmaniosis, the expected Index value is 2.64.

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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