LEISHMANIA INFANTUM

Enzyme immunoassay for the diagnosis of visceral leishmaniasis

96 assays on individual wells

Technical sheet and instructions for article N° 9500, EC reg. N°: H-CH/CA01/IVD/01756

Intended use:

Serological diagnosis (IgG) of visceral leishmaniasis. Sero-epidemiological surveys. Serological controle of HIV patients from endemic areas. Post therapy controle of HIV-Leishmania co-infected patients.

Principle and presentation:

The kit provides the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on microtitration wells sensitized with ***Leishmania infantum*** soluble promastigote antigens. The presence of parasite specific serum antibodies is detected with proteine A-alkaline phosphatase conjugate. Sensitized wells are provided as breakable strips for the economical assay of small series of samples.

Material contained in the kit (96 assays):

9500-1	Breakable ELISA strips sensitized with ***Leishmania infantum*** soluble antigens	96	wells
9500-2	TBS-Tween (10 x) concentrate	50	ml
9500-3	Washing solution (10 x) concentrate	50	ml
9500-4	Enzyme buffer	50	ml
9500-5	Stopping solution (K_3PO_4)	25	ml
9500-6	Negative control serum	200	µl
9500-7	Weak positive serum (cut off)	200	µl
9500-8	Positive control serum	200	µl
9500-9	Protein A-alkaline phosphatase conjugate	300	µl
9500-10	Phosphatase substrate	20	tablets
9500-11	Multipipette reservoir, 25 ml	1	piece
9500-12	Frame for ELISA 8-well holder	1	piece
9500-13	Technical sheet and instructions		

Shelf life and storage:

Store kit at 2° to 8° C. The expiry date of the kit is printed on the side of the box.

Equipment needed but not provided with the kit:

Pipettes (ml and µl). Flasks. Tubes for the dilution of sera. Adhesive tape to cover wells during incubations. Distilled water. Incubator set at 37° C. ELISA reader set at 405 nm.

Preparation of reagents before use:

ELISA wells: open side of aluminum bag N° 1 and remove number of wells needed. Place sensitized wells in 8-well holder(s). Insert holder(s) in the frame in the correct orientation. Reseal open package with desiccant pad.

TBS-Tween solution (TBS-Tw): dilute TBS-Tw (10 x) concentrate N° 2, in distilled water.

Washing solution: dilute washing solution (10 x) concentrate N° 3, in distilled water. You may also use your own washing solution. Buffers containing phosphate should be avoided in this solution.

Negative, weak positive and positive **control sera**: dilute 10 µl control sera N° 6 to 8 in 190 µl TBS-Tw solution (dil. 1/20).

Sera to be tested: dilute 10 µl serum in 2.0 ml TBS-Tw solution (dil. 1/201).

Protein A-alkaline phosphatase **conjugate**: dilute conjugate N° 9 in TBS-Tw solution (dil. 1/51).

Substrate solution: prewarm undiluted enzyme buffer N° 4 at ambient temperature.
Before the addition of substrate to the ELISA wells, dissolve tablet(s) of phosphatase substrate N° 10 in buffer N°4 (1 tablet in 2.5 ml buffer). Mix well after dissolution of the tablet(s).

Stopping solution: use reagent N° 5 undiluted.

Warning : Solutions 9500-02, 9500-03, 9500-04 and 9500-09 contain respectively 0.1%,0.02%,0.01% and 0.1% of sodium azide (N_aN_3), this substance is toxic. The stopping solution, 9500-5 (0.5 M K_3PO_4) is irritant.

The negative, weak positive, and positive control sera of the kit (6 to 8) are of canine origin.

Volumes to be prepared:

			Total number of wells to be used			
			3-4	**5-6**	**7-8**	**9-10**
TBS-Tween (10 x)	N°2 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Washing solution (10 x)	N°3 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Conjugate	N°9 + TBS-Tw	µl + µl	10 + 500	15 + 750	20 + 1000	25 + 1250
Control sera	N° 6-8 +TBS-Tw	µl + µl	10 + 190	10 + 190	10 + 190	10 + 190
Sera to be tested	Serum + TBS-Tw	µl + µl	10 + 2000	10 + 2000	10 + 2000	10 + 2000
Substrate	N°10 + N°4	tabl. + ml	1 + 2.5	1 + 2.5	1 + 2.5	1 + 2.5

Procedure:

Step 1: Blocking:

Fill completely wells with TBS-Tween (TBS-Tw).

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

Remove TBS-Tw by aspiration or by shaking the strips over the sink.

Step 2: Incubation with serum samples:

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

Fill the subsequent three wells with 100 µl diluted negative, weak positive (cut off) and positive control sera respectively (100 µl each).

Fill remaining wells with the diluted sera to be tested (100 µl each).

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

Remove sera and wash 4 x with washing solution.

Step 3: Incubation with conjugate:

Distribute 100 µl diluted Protein A-alkaline phosphatase conjugate in each well.

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

Remove conjugate and wash 4 x with washing solution.

Step 4: Incubation with substrate:

Distribute 100 µl substrate solution per well.

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

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

Step 5: Measurement of absorbances:

Wipe bottom of wells, eliminate bubbles and measure absorbances at 405 nm.

Interpretation:

Subtract value of the no-serum blank from all measured values. The test is valid if the following criteria are met: absorbance(A) of positive control > 1.200, A of négative control < 10% of A of positive control, A of blank againdt air < 0.350.

The titer of the weak positive (cut off) serum N° 7 has been set to discriminate optimally between sera of clinically documented cases of leishmaniasis and normal human sera .

The result is **negative** when the absorbance of the analyzed sample is lower than the absorbance of the weak positive serum. In this case, the titer of IgG antibody against *Leishmania* soluble antigens is clinically non-significant.

The result is **positive** when the absorbance of the analyzed sample is higher than the absorbance of the weak positive serum. In this case, the titer of IgG antibody against *Leishmania* soluble antigens is usually clinically significant.

Sensitivity and specificity of the assay:

The sensitivity of the test is better than 95% in immunocompetents patients suffuring from visceral leishmaniasis du to *L.infantum*. For HIV-*Leishmania* co-infected patients the sensitivity of the test is between 60 and 85%. 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*.
The specificity of the test is better than 95% with sera from Swiss blood donors. Crossreactivity might occur in some other parasitic infections such as african trypanosomiasis, Chagas disease and cutaneous and mucocutaneous leishmaniasis. Internal evaluation showed that hemorragic, lipemic or icteric sera do not interfere with the results of the test.

Using the assay for dogs:

Dog sera can be tested with this kit in the same conditions.

References:

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