TOXOCARA CANIS

Enzyme immunoassay for the diagnosis of human toxocarosis

96 assays on individual wells

Technical sheet and instructions for article N° 9200, EC reg. N°: H-CH/CA01/IVD/01755

Intended use:

Serological diagnosis (IgG) of human toxocarosis (visceral or ocular *larva migrans* syndrome). Confirmation of suspected clinical cases and sero-epidemiological surveys.

Principle and presentation:

The kit provides the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on microtitration wells sensitized with ***Toxocara canis*** E/S larval antigens. The presence of parasite specific serum antibodies is detected with anti-human IgG-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):

9200-1	Breakable ELISA strips sensitized with ***Toxocara canis*** E/S antigens	96	wells
9200-2	TBS-Tween (10 x) concentrate	50	ml
9200-3	Washing solution (10 x) concentrate	50	ml
9200-4	Enzyme buffer	50	ml
9200-5	Stopping solution (K_3PO_4)	25	ml
9200-6	Negative control serum	200	µl
9200-7	Weak positive serum (cut off)	200	µl
9200-8	Positive control serum	200	µl
9200-9	Anti-human IgG-alkaline phosphatase conjugate	300	µl
9200-10	Phosphatase substrate	20	tablets
9200-11	Multipipette reservoir, 25 ml	1	piece
9200-12	Frame for ELISA 8-well holder	1	piece
9200-13	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).

Anti-human IgG-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 9200-02, 9200-03, 9200-04 and 9200-09 contain respectively 0.1%, 0.1%, 0.01% and 0.1% of sodium azide (N_aN_3). Solution 9200-2 contain 0.02% of merthiolate. These substances are toxic. The stopping solution, 9200-5 (0.5 M K_3PO_4) is irritant.
The negative, weak positive and positive control sera of the kit (N° 6 to 8) have been tested for anti-HIV 1, for anti-HIV 2 and for anti-HVC antibodies and for HBs antigens and found negative.

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 solution	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 for 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 for 30 minutes at 37° C.

Remove sera and wash 4 x with washing solution.

Step 3: Incubation with conjugate:

Distribute 100 µl diluted anti-human IgG-alkaline phosphatase conjugate in each well.

Cover wells with adhesive tape and incubate for 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 negative control < 8 % of A of positive control, A of blank against air < 0.300.

The antibody concentration of the weak positive (cut off) serum N° 7 has been set to discriminate optimally between sera of clinically documented cases of toxocarosis 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 N° 7. In this case, the IgG antibody concentration against *Toxocara* E/S 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 control. In this case, the IgG antibody concentration against *Toxocara* E/S antigens is usually clinically significant.

Sensitivity and specificity of the assay:

The diagnostic sensitivity of the test is 91 %. The specificity of the reaction with regards to other parasitic infections is 86%. Crossreactivity mainly occur in patients with Trichinellosis, fascioliasis, amebiasis and stongyloidosis. A specificity of 96% was found with 500 sera of blood donnors (Swiss). A detailed evaluation of the kit has been published by Jacquier *et al*. (1991). Internal evaluation showed that hemorragic, lipemic or icteric sera do not interfere with the results of the test.

References:

De Savigny, D.H., Voller, A. and Woodruff, A.W. (1979) Toxocariasis: serological diagnosis by enzyme immunoassay. J. Clin. Path. **32 :** 284-288.

Speiser, F.and Gottstein, B. (1984) A collaborative study on larval excretory/secretory antigens of *Toxocara canis* for the immunodiagnosis of human toxocariasis with ELISA. Acta Tropica. **41:** 361-372.

Glickmann, L.T., Schantz, P.M. and Grieve, R.B. (1986) Toxocariasis. In: Immunodiagnosis of Parasitic Diseases, Walls K.W. & Schantz P.M. éditors, Academic Press, pp 201-231.

Gillespie, S.H. (1988) The épidemiology of *Toxocara canis*. Parasitology Today. **4 :** 180-182.

Jacquier, P., Gottstein, B., Singelin, Y. and Eckert, J. (1991) Immunodiagnosis of Toxocarosis in Humans: Evaluation of a New Enzyme-Linked Immunosorbent Assay Kit. J. Clin. Microbiol. **29**, 1831-1835.

Wolfrom, E., Chêne, G., Boisseau, H., Beylot, C., Géniaux, M. and Taïeb, A.. (1995) Chronic urticaria and *Toxocara canis.* The Lancet. **345:** 192.

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