ACANTHOCHEILONEMA VITEAE

Enzyme immunoassay for the diagnosis of human filariosis

96 assays on individual wells

Technical sheet and instructions for article N° 9400,EC reg. N°:H-CH/CA01/IVD/01755

Intended use:

Screening of suspects clinical cases and routine serology (IgG) of human filarial infections including lymphatique and african filariasis (Bancroftian and Malayan filariosis, Loaosis, Onchocercosis and Mansonellosis).

Principle and presentation:

The kit provides the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on microtitration wells sensitized with *Acanthocheilonema viteae* somatic antigens. The presence of IgG antibody in serum is detected with a 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):

9400-1	Breakable ELISA strips sensitised with ***Acanthocheilonema viteae*** somatic antigens	96	wells
9400-2	TBS-Tween (10 x) concentrate	50	ml
9400-3	Washing solution (10 x) concentrate	50	ml
9400-4	Enzyme buffer	50	ml
9400-5	Stopping solution (K_3PO_4)	25	ml
9400-6	Negative control serum	200	µl
9400-7	Weak positive serum	200	µl
9400-8	Positive control serum	200	µl
9400-9	Proteine A-alkaline phosphatase conjugate	300	µl
9400-10	Phosphatase substrate	20	tablets
9400-11	Multipipette reservoir, 25 ml	1	piece
9400-12	Frame for ELISA 8-well holder	1	piece
9400-13	Technical sheets 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 aluminium 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 dessicant 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).

Proteine 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: K_3PO_4 solutions may be irritant: Use caution.

Warning : Solutions 9400-02, 9400-03, 9400-04 and 9400-09 contain respectively 0.1%, 0.1%, 0.01% and 0.1% of sodium azide (N_aN_3). Solution 9400-2 contain 0.02% of merthiolate. These substances are toxic. The stopping solution, 9400-5 (0.5 M K_3PO_4) is irritant.
The negative, weak positive, and positive control sera of the kit (6 to 8) are from rabbits.

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 proteine 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 negative control < 12% of A of positive control, A of blank against 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 filarioses 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 titer of IgG antibody against *Acanthocheilonema viteae* somatic antigens is clinically non-significant. If filariasis is strongly suspected, patients sould be examined for microfilariae. Négative sérology may be observed in patients with microfilaremia.

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 titer of IgG antibody against *Acanthocheilonema viteae* somatic antigens is usually significant. This result should be considered with regards to the endemic situation and clinical symptoms.

Sensitivity and specificity of the assay :

The sensitivity and the specificity of the test for human filarioses are respectively of 95% and 98 %. This test does not differentiate between the different filarial infections. It is used as a first screening method.
Cross-reactivity often occur with antibodies to other parasites such as *Ascaris*, *Trichinella*, *Ancylostoma*, *Fasciola hepatica* et *Echinococcus granulosus*. Positive results have to be interpreted in relation to the endemic and clinical background.
The kit has been evaluated by an independent laboratory : Out of 22 sera from positive patients for filariasis (patients with microfilaremia and/or with positive serology with other techniques and an epidemiological and clinical background of filarasis) 21 sera were positifs by this test. Internal evaluation showed that hemorragic, lipemic or icteric sera do not interfere with the results of the test.

References:

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