ECHINOCOCCUS MULTILOCULARIS

Enzyme immunoassay for the diagnosis of human alveolar echinococcosis

96 assays on individual wells

Technical sheet and instructions for article N° 9300, EC reg. N°: H-CH/CA01/IVD/01757

Intended use:

Serological diagnosis (IgG) of human alveolar echinococcosis (alveolar hydatid disease). Sero-epidemiological surveys and examination of persons at risk, following exposition to infection. Post-operative control.

Principle and presentation:

The kit provides the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on microtitration wells sensitized with ***Echinococcus multilocularis*** Em2-Em18 antigens. The presence of IgG antibody in serum is detected with a Protein 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):

9300-01	Breakable ELISA strips sensitized with ***Echinococcus multilocularis*** Em2-Em18 antigens	96	wells
9300-02	TBS-Tween (10 x) concentrate	50	ml
9300-03	Washing solution (10 x) concentrate	50	ml
9300-04	Enzyme buffer	50	ml
9300-05	Stopping solution (K_3PO_4)	25	ml
9300-06	Negative control serum	200	µl
9300-07	Weak positive (cut off) serum	200	µl
9300-08	Positive control serum	200	µl
9300-09	Protein A-alkaline phosphatase conjugate	300	µl
9300-10	Phosphatase substrate	20	tablets
9300-11	Multipipette reservoir 25 ml	1	piece
9300-12	Frame for ELISA 8-well holder	1	piece
9300-13	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 aluminum bag 9300-01 at its side and remove number of wells needed. 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.

TBS-Tween solution (TBS-Tw): dilute TBS-Tw (10 x) concentrate 9300-02, 1/10 in distilled water.

Washing solution: dilute washing solution (10 x) concentrate 9300-03 in distilled water. You may also use your own washing solution. Buffers containing phosphate should be avoided in this solution.

Negative, weak positive (cut off) and positive **control sera**: dilute 10 µl control sera 9300-06, -07 and -08 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 9300-09, 1/51 in TBS-Tw solution.

Substrate solution: prewarm undiluted enzyme buffer 9300-04 at ambient temperature. Before the addition of substrate to the ELISA wells, dissolve the required number of phosphatase substrate tablets 9300-10 in undiluted buffer 9300-04 (1 tablet in 2.5 ml buffer). Mix well after dissolution of the tablet(s).

Stopping solution: use reagent 9300-05 undiluted.

Warning : Solutions 9300-02, 9300-03, 9300-04 and 9300-09 contain respectively 0.1%, 0.05%, 0.01% and 0.1% of sodium azide (N_aN_3). Solution 9300-02 contain 0.02% of merthiolate. These substances are toxic. The stopping solution, 9300-05 (0.5 M K_3PO_4) is irritant. Control sera 9300-06, -07 and -08 are from rabbit.

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
Protein A 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 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 protein A-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.

Results validation and interpretation:

The test is valid if the absorbance (A) of the TBS-Tw sample (no serum blank) is < 0.350. After subtraction of this blank, the absorbance of the positive control should be > 1.000 and the absorbance of the negative control should be < 10 % of the positive control.

The antibody concentration of the weak positive (cut off) serum has been set to discriminate optimally between sera of cases of alveolar echinococcosis and normal human sera.

A sample with an absorbance lower than the weak positive control (cut off) serum has a non-significant antibody concentration against ***Echinococcus multilocularis*** Em2-Em18 antigens, it is therefore serologically **negative**.

A sample with an absorbance higher than the weak positive (cut off) control serum is serologically **positive**.

Sensibility and spécificity:

A diagnostic sensitivity of 93 % was found on a group of 27 patients with alveolar echinococcosis (*Echinococcus multilocularis*). Approximately 85 % (n = 19) of cystic echinococcoses (*E. granulosus*) are negative with this test.

The specificity of the assay with sera from patients with other parasitoses was tested. Results were negative with 90 % of patients with other helminthiases (n=51) and 74 % with protozooses (n = 23). 158 sera of blood donors (Swiss) were negative at 98 %. Internal evaluation showed that hemorragic, lipemic or icteric sera do not interfere with the results of the test

References:

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