ECHINOCOCCUS GRANULOSUS

Enzyme immunoassay for the diagnosis of human echinococcoses

96 assays on individual wells

Technical sheet and instructions for article N° 9350, EC reg. N°: H-CH/CA01/IVD/01757

Intended use:

Serological diagnosis (IgG) of human cystic hydatid disease (caused by ***Echinococcus granulosus***). This assay can also be used for the serological diagnosis of alveolar hydatid disease (caused by ***Echinococcus multilocularis***). Positive and doubtful cases should be retested with the *Echinococcus multilocularis*-specific Em2-Em18 -ELISA (Bordier Affinity Products, article N° 9300) to identify the infecting *Echinococcus* species.

Principle and presentation:

The kit provides the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on microtitration wells sensitized with ***Echinococcus granulosus*** hydatid fluid antigen. 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):

9350-01	Breakable ELISA strips sensitised with ***Echinococcus granulosus*** antigen (hydatid fluid)	96	wells
9350-02	TBS-Tween (10 x) concentrate	50	ml
9350-03	Washing solution (10 x) concentrate	50	ml
9350-04	Enzyme buffer	50	ml
9350-05	Stopping solution (K_3PO_4)	25	ml
9350-06	Negative control serum	200	µl
9350-07	Weak positive (cut off) serum	200	µl
9350-08	Positive control serum	200	µl
9350-09	Protein A-alkaline phosphatase conjugate	300	µl
9350-10	Phosphatase substrate	20	tablets
9350-11	Multipipette reservoir 25 ml	1	piece
9350-12	Frame for ELISA 8-well holder	1	piece
9350-13	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 9350-01 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 9350-02, 1/10 in distilled water.

Washing solution: dilute washing solution (10 x) concentrate 9350-03, 1/10 in distilled water. You may also use your own washing solution but buffers containing phosphate should be avoided.

Negative, weak positive (cut off) and positive **control sera**: dilute 10 µl control sera 9350-06, -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 9350-09, 1/51 in TBS-Tw solution.

Substrate solution: prewarm undiluted enzyme buffer 9350-04 at ambient temperature. Before the addition of substrate to the ELISA wells, dissolve the required number of phosphatase substrate tablets 9350-10 in undiluted buffer 9350-04 (1 tablet in 2.5 ml buffer). Mix well after dissolution of the tablet(s).

Stopping solution: use reagent 9350-05 undiluted.

Warning : Solutions 9350-02, 9350-03, 9350-04 and 9350-09 contain respectively 0.1%, 0.05%, 0.01% and 0.1% of sodium azide (N_aN_3). Solution 9350-02 contain 0.02% of merthiolate. These substances are toxic. The stopping solution, 9350-05 (0.5 M K_3PO_4) is irritant. Control sera 9350-06, -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° 02 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Washing solution (10 x)	N° 03 + H_2O	ml + ml	1 + 9	2 + 18	3 + 27	4 + 36
Protein A conjugate	N° 09 + 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° 04	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 > 1.200 and the absorbance of the negative control should be < 12 % of the positive control.

The antibody concentration of the cut off serum 9350-07 has been set to discriminate optimally between sera of cases of cystic hydatid disease and sera of healthy individuals.

The result is **negative** when the absorbance of the analysed serum is lower than the absorbance of the cut off serum. In this case, the antibody concentration against the *E. granulosus* antigen is not considered as significant.

The result is **positive** when the absorbance of the analysed serum is higher than the absorbance of the cut off serum. In this case, the IgG antibody concentration against the *E. granulosus* antigen is considered as significant.

Sensitivity and specificity of the assay:

A diagnostic sensitivity of 96 % was found on a group of 24 patients with cystic hydatid disease (*Echinococcus granulosus*). Most patients (90 %) with alveolar hydatid disease (*Echinococcus multilocularis*) were also found positive with this assay.

The specificity of the assay with sera from patients with other parasitoses were tested. Results were negative with 82% of patients with helminthiases (n=51) and 83% with protozooses (n=23). 150 sera of blood donors (Swiss) were negative at 97 %. Internal evaluation showed that hemorragic, lipemic or icteric sera do not interfere with the results of the test

Within the usual range of samples analyzed in a European laboratory, the predictive value of a negative result is close to 100 %. In contrast, a positive result has to be confirmed in all cases with more specific assays (Em2-Em18 –ELISA and search for anti-8 Kd antibodies by immunoblot).

References:

Gottstein, B. (1992) Molecular and Immunological diagnosis of Echinococcosis. Clin. Microbiol. Rev. **5 :** 248-261.

Gottstein, B., Jacquier, P., Bresson-Hadni, S. and Eckert, J. (1993) Improved primary immunodiagnosis of Alveolar Echinococcosis in humans by an enzyme-linked immunosorbent assay using the Em2 ^{plus} antigen. J. Clin. Microbiol. **31:** 373-376.

Poretti, D., Felleisen, E., Grimm, F., Pfister, M., Teuscher, F., Zuercher, C., Reichen, J. and Gottstein, B. (1999) Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. Am. J. Trop. Med. Hyg. **60:** 193-198.

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