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):

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>9300-01</td>
<td>Breakable ELISA strips sensitized with <em>Echinococcus multilocularis</em> Em2-Em18 antigens</td>
<td>96 wells</td>
</tr>
<tr>
<td>9300-02</td>
<td>TBS-Tween (10 x) concentrate</td>
<td>50 ml</td>
</tr>
<tr>
<td>9300-03</td>
<td>Washing solution (10 x) concentrate</td>
<td>50 ml</td>
</tr>
<tr>
<td>9300-04</td>
<td>Enzyme buffer</td>
<td>50 ml</td>
</tr>
<tr>
<td>9300-05</td>
<td>Stopping solution (K$_3$PO$_4$)</td>
<td>25 ml</td>
</tr>
<tr>
<td>9300-06</td>
<td>Negative control serum</td>
<td>200 µl</td>
</tr>
<tr>
<td>9300-07</td>
<td>Weak positive (cut off) serum</td>
<td>200 µl</td>
</tr>
<tr>
<td>9300-08</td>
<td>Positive control serum</td>
<td>200 µl</td>
</tr>
<tr>
<td>9300-09</td>
<td>Protein A-alkaline phosphatase conjugate</td>
<td>300 µl</td>
</tr>
<tr>
<td>9300-10</td>
<td>Phosphatase substrate</td>
<td>20 tablets</td>
</tr>
<tr>
<td>9300-11</td>
<td>Multipipette reservoir 25 ml</td>
<td>1 piece</td>
</tr>
<tr>
<td>9300-12</td>
<td>Frame for ELISA 8-well holder</td>
<td>1 piece</td>
</tr>
<tr>
<td>9300-13</td>
<td>Technical sheet and instructions</td>
<td></td>
</tr>
</tbody>
</table>

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:


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 (N₃Na₃).  Solution 9300-02 contain 0.02% of merthiolate.  These substances are toxic.  The stopping solution, 9300-05 (0.5 M K₃PO₄) is irritant.  Control sera 9300-06, -07 and -08 are from rabbit.

### Volumes to be prepared:

<table>
<thead>
<tr>
<th></th>
<th>Total number of wells to be used</th>
<th>3-4</th>
<th>5-6</th>
<th>7-8</th>
<th>9-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBS-Tween (10 x)</td>
<td>N°2 + H₂O</td>
<td>ml + ml</td>
<td>1 + 9</td>
<td>2 + 18</td>
<td>3 + 27</td>
</tr>
<tr>
<td>Washing solution (10 x)</td>
<td>N°3 + H₂O</td>
<td>ml + ml</td>
<td>1 + 9</td>
<td>2 + 18</td>
<td>3 + 27</td>
</tr>
<tr>
<td>Protein A conjugate</td>
<td>N°9 + TBS-Tw</td>
<td>µl + µl</td>
<td>10 + 500</td>
<td>15 + 750</td>
<td>20 + 1000</td>
</tr>
<tr>
<td>Control sera</td>
<td>N° 6-8 +TBS-Tw</td>
<td>µl + µl</td>
<td>10 + 190</td>
<td>10 + 190</td>
<td>10 + 190</td>
</tr>
<tr>
<td>Sera to be tested</td>
<td>Serum + TBS-Tw</td>
<td>µl + µl</td>
<td>10 + 2000</td>
<td>10 + 2000</td>
<td>10 + 2000</td>
</tr>
<tr>
<td>Substrate</td>
<td>N°10 + N°4</td>
<td>tabl. + ml</td>
<td>1 + 2.5</td>
<td>1 + 2.5</td>
<td>1 + 2.5</td>
</tr>
</tbody>
</table>
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:


