HYDATIDOSIS ELISA IgG

G1006: Indirect immunoenzyme assay to test IgG antibodies against *Echinococcus granulosus* in human serum. 96 tests.

INTRODUCTION:

*Echinococcus granulosus* is the causal agent of human hydatidosis. Hydatid cysts are most often located in liver, preferably in the right lobe. The intensity of the immune response depends on the location and integrity of the cyst. Cysts in liver and bone are more reactive than those in lung, brain or spleen. The most frequently used techniques for the diagnosis of hydatidosis are Casoni’s skin test, complement fixation, indirect hemagglutination, immunofluorescence and ELISA. The ELISA, with hydatid fluid of sheep origin as antigen, is widely used for the diagnosis of the disease, showing a high sensitivity for hepatic cysts, although lower for pulmonary locations. Cross-reactions may appear in patients infected by other helminths (mainly with cysticercosis) and in oncological processes.

PRINCIPLE OF THE TEST:

The ELISA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a substrate solution (TMB) to render a blue coloured soluble product which turns into yellow after adding the acid stopping solution.

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KIT FEATURES:

All reagents, except for the washing solution, are supplied ready to use. Serum dilution solution and conjugate are coloured to help in the performance of the technique. Sample predilution is not necessary. Break-apart individual wells are supplied, so that the same number of wells is consumed than the number of tests performed.

KIT CONTENTS:

1 VIRCELL HYDATIDOSIS PLATE: 1 96-wells plate coated with *Echinococcus granulosus* antigen.
3 VIRCELL IgG POSITIVE CONTROL: 500 µl of positive control serum containing Proclin.
4 VIRCELL IgG CUT OFF CONTROL: 500 µl of cut off control serum containing Proclin.
5 VIRCELL IgG NEGATIVE CONTROL: 500 µl of negative control serum containing Proclin.
6 VIRCELL IgG CONJUGATE: 15 ml of anti-human IgG peroxidase conjugate dilution in an orange-coloured Proclin-containing buffer. Ready to use.
7 VIRCELL TMB SUBSTRATE SOLUTION: 15 ml of substrate solution containing tetramethylbenzidine (TMB). Ready to use.
8 VIRCELL STOP REAGENT: 15 ml of stopping solution: 0.5 M sulphuric acid.
9 VIRCELL WASH BUFFER: 50 ml of 20x washing solution: a phosphate buffer containing Tween80 and Proclin.

STORAGE REQUIREMENTS:

Store at 2-8°C and check expiration date.

STORAGE OF REAGENTS ONCE OPENED:

1x washing solution 4 months at 2-8°C
Rest of reagents Refer to package label for expiration date (at 2-8°C)

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.

Do not let the plate dry between washing and reagent addition.

Substrate solution is light sensitive. Avoid light exposure and discard if blue colour develops during storage. Substrate solution should not get in contact with oxidizers such as bleach solutions or metals. Make sure that no metal components come in contact with the substrate.

Use only the amount of washing, serum dilution, conjugate and TMB solutions required for the test. Do not return the excess solution into the bottles.

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For *in vitro* diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only the serum dilution, washing, stopping and substrate solutions are compatible with the equivalents in other VIRCELL ELISA references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Do not use in the event of damage to the package.
5. Never pipette by mouth.
6. Serum dilution solution, plate, conjugates and controls in this kit include substances of animal origin. Controls include as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human
ASSAY PROCEDURE:

1. Set incubator/water bath to 37±1ºC.
2. Bring all reagents to room temperature before use (approximately 1 hour) before diluting. Once diluted, store at 2-8ºC.
3. Shake all components.
4. Before incorporating this product onto an automatic processing system, we strongly recommend the performance of a pre-evaluation assay. To this purpose, VIRCELL counts with sets of samples reserved for evaluation in parallel with the manual technique. These sets of samples are available on request, as well as a list of commercial systems which have already been validated for use with the VIRCELL ELISA range.

5. Add 100 µl of serum diluent to all wells. Add 5 µl of each sample, 5 µl of positive control, 5 µl of cut off control (in duplicate) and 5 µl of negative control into the corresponding wells. If the assay is performed manually, shake the plate in a plate shaker (2 min) in order to achieve a homogeneous mixture of the reagents. If for some reason correct shaking cannot be guaranteed, a pre-dilution of the sample in a separate tube or plate should be made, using double volume of serum diluent and sample. Mix homogeneously with the pipette and dispense 105 µl of each diluted sample to the wells.

6. Cover with a sealing sheet and incubate at 37±1ºC for 45 min.
7. Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution per well. Drain off any remaining liquid.
8. Immediately add 100 µl of IgG conjugate solution into each well.
9. Cover with a sealing sheet and incubate in incubator/water bath at 37±1ºC for 30 min.
10. Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution per well. Drain off any remaining liquid.
11. Immediately add 100 µl of substrate solution into each well.
12. Incubate at room temperature for 20 minutes protected from light.
13. Add immediately 50 µl of stopping solution into all wells.
14. Read with a spectrophotometer at 450/620 nm within 1 hour of stopping.

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available. The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:

Positive, negative and cut off controls must be run with each test run. It allows the validation of the assay and kit.

Optical densities (O.D.) must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

<table>
<thead>
<tr>
<th>CONTROL</th>
<th>O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE CONTROL</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>NEGATIVE CONTROL</td>
<td>&lt;0.55</td>
</tr>
<tr>
<td>CUT OFF CONTROL</td>
<td>&lt;0.7 x(O.D. POSITIVE CONTROL)</td>
</tr>
<tr>
<td></td>
<td>&gt;1.5 x(O.D. NEGATIVE CONTROL)</td>
</tr>
</tbody>
</table>

INTERPRETATION OF RESULTS:

Calculate the mean O.D. for cut off serum.

Antibody index=(sample O.D./cut off serum mean O.D.) x 10

<table>
<thead>
<tr>
<th>INDEX</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>Negative</td>
</tr>
<tr>
<td>9-11</td>
<td>Equivocal</td>
</tr>
<tr>
<td>&gt;11</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Samples with equivocal results must be restested and/or a new sample obtained for confirmation.

Samples with indexes below 9 are considered as not having IgG specific antibodies against E. granulosus.

Samples with indexes above 11 are considered as having IgG specific antibodies against E. granulosus.

LIMITATIONS:

1.-This kit is intended to be used with human serum.
2.-The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3.-The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4.-This test will not indicate the site of infection. It is not intended to replace isolation.
5.-Lack of significant rise in antibody level does not exclude the possibility of infection.
6.-Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
7.-Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
8.-The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.

FOR IN VITRO DIAGNOSTIC USE

Manufacturer: VIRCELL, S.L. Pza. Domínguez Ortiz 1. Polígono Industrial Dos de Octubre. 18320 Santa Fe *GRANADA* SPAIN* Tel.+34.958.441264* Fax+34.958.510712
http://www.vircell.com
PERFORMANCE

SENSITIVITY AND SPECIFICITY:

329 serum samples were assayed with HYDATIDOSIS ELISA IgG against an indirect haemagglutination test. The results were as follows:

<table>
<thead>
<tr>
<th>IgG</th>
<th>SAMPLE N</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>329</td>
<td>97%</td>
<td>96%</td>
</tr>
</tbody>
</table>

Indeterminate values were omitted from the final calculations.

INTRA-ASSAY PRECISION:

3 sera were individually pipetted 10 times each serum in a single assay performed by the same operator in essentially unchanged conditions. The results were as follows:

<table>
<thead>
<tr>
<th>SERUM</th>
<th>N</th>
<th>% C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>10</td>
<td>1.98</td>
</tr>
<tr>
<td>NC</td>
<td>10</td>
<td>6.67</td>
</tr>
<tr>
<td>CO</td>
<td>10</td>
<td>2.52</td>
</tr>
</tbody>
</table>

C.V. Coefficient of variation

INTER-ASSAY PRECISION:

3 sera were individually pipetted on 5 consecutive days by 2 different operators. The results were as follows:

<table>
<thead>
<tr>
<th>SERUM</th>
<th>N</th>
<th>% C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>10</td>
<td>2.78</td>
</tr>
<tr>
<td>NC</td>
<td>10</td>
<td>6.20</td>
</tr>
<tr>
<td>CO</td>
<td>10</td>
<td>4.20</td>
</tr>
</tbody>
</table>

C.V. Coefficient of variation

CROSS REACTIVITY AND INTERFERENCES:

8 samples known to be positive for other specimens of the taxonomic group (Leishmania infantum, Taenia solium, Trichinella spiralis, Toxoplasma gondii), were assayed.

The negative results of the test demonstrated the specific reaction of the kit with no cross-reaction or interferences with the referred specimens. Cross-reactions may appear in patients infected by other helminths (mainly with cysticercosis) and in oncological processes.

SYMBOLS USED IN LABELS: