HELICOBACTER PYLORI ELISA
IgA
For in vitro diagnostic use

A1022: Indirect immunoenzymatic assay for detection and semiquantification of IgA antibodies against Helicobacter pylori in human serum/plasma. 96 tests.

INTRODUCTION:
Helicobacter pylori has a worldwide distribution and a high prevalence. The infection with H. pylori is well established as a major cause of gastric and duodenal ulcers. The persistent infection with H. pylori is a risk factor for the development of gastric carcinoma and lymphoma. The infection produces elevated levels of specific H. pylori IgG and IgA antibodies in serum. IgM specific levels has not proven useful in the clinical laboratory. ELISA tests for the detection of H. pylori antibodies are sensitive, specific and cost effective in untreated patients. The detection of H. pylori specific IgA alone is less sensitive than the detection of specific IgG antibodies. In untreated persons specific IgG and IgA remain elevated for years and successful eradication decreases the IgG and IgA levels, although in some individuals specific antibodies can persist during a long time. Because the infection with H. pylori is so prevalent the test should be performed only on individuals with symptoms. The prevalence of H. pylori antibodies increases with the age. A positive result only indicates that the patient has antibodies to H. pylori and if the individual has not been treated, a positive result very likely indicates an active infection with H. pylori. A definitive diagnosis should be given only when the clinical signs and symptoms of the patient are compatible.

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.

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:
- VIRCELL HELICOBACTER PYLORI PLATE: 1 96-wells plate coated with detergent-soluble antigens of H. pylori, strain 26695.
- VIRCELL IgA POSITIVE CONTROL: 500 µl of positive control serum containing 200 U./ml of IgA anti-Helicobacter pylori, containing Neolone and Bronidox.
- VIRCELL IgA CUT OFF CONTROL: 500 µl of cut off control serum containing 10 U./ml of IgA anti-Helicobacter pylori, containing Neolone and Bronidox.
- VIRCELL IgA NEGATIVE CONTROL: 500 µl of negative control serum containing Neolone and Bronidox.
- VIRCELL IgA CONJUGATE: 15 ml of anti-human IgA peroxidase conjugate dilution in an orange-coloured Neolone and Bronidox-containing buffer. Ready to use.
- VIRCELL TMB SUBSTRATE SOLUTION: 15 ml of substrate solution containing tetramethylbenzidine (TMB). Ready to use.
- VIRCELL STOP REAGENT: 15 ml of stopping solution: 0.5 M sulphuric acid.
- VIRCELL WASH BUFFER: 50 ml of 20x washing solution: a phosphate buffer containing Tween®-20 and Proclin.
- VIRCELL SEMIQUANTIFICATION SAMPLE CONTROL: 500 µl of semiquantification sample control, containing 20-50 U./ml of IgA anti- Helicobacter pylori, containing Neolone and Bronidox.

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

Materials required but not supplied:
- Eight channel micropipette 100 µl.
- ELISA plate washer.
- Thermostatized incubator/water bath.
- ELISA plate spectrophotometer with a 450 nm measuring filter and a 620 nm reference filter.
- Alternatively, an ELISA automated processor.
- Distilled water.
- Human IgG sorbent (ref. Vircell S001).

STORAGE REQUIREMENTS:
Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

STORAGE OF REAGENTS ONCE OPENED:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x washing solution</td>
<td>4 months at 2-8°C</td>
</tr>
<tr>
<td>Rest of reagents</td>
<td>Refer to package label for expiration date (at 2-8°C)</td>
</tr>
</tbody>
</table>

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 human 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 Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. The wells are coated with inactivated H. pylori antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that these or other infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
7. Substrate solution may be irritant to skin and mucous. In case of contact with this solution, rinse thoroughly with water and seek medical attention. For further information a Material Safety Data Sheet is available.
8. 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.
9. During incubation times, an adequate sealing of the plates with the adhesive film included in the kit avoids the desiccation of the samples, and guarantees the repeatability of the results.
10. This product has been designed for exclusive use in conjunction with VIRCELL human IgG sorbent (Vircell ref. S001).
11. Avoid contact of Stop Solution (0.5 M sulfuric acid) with skin or eyes. If contact occurs, immediately flush the area with water.

SPECIMEN COLLECTION AND HANDLING:
Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum/plasma samples to be are refrigerated (2-8°C) upon collection or frozen (-20°C) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sample. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PRELIMINARY PREPARATION OF THE REAGENTS:
Only the washing solution must be prepared in advance. Fill 50 ml of 20x washing solution up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37°C before diluting. Once diluted, store at 2-8°C.

ASSAY PROCEDURE:
1. Set incubator/water bath to 37±1°C.
2. Bring all reagents to room temperature before use (approximately 1 hour), without removing the plate from the bag.
3. Shake all components.
4. Remove the plate from the package. Determine the numbers of wells to be employed counting in four wells for the controls: two for the cut off serum and one each for the negative and positive sera. Wells not required for the test should be returned to the pouch, which should then be sealed.
5. Add 25 µl of VIRCELL IgG sorbent (ref. S001) to each of the required wells, except for the wells where controls will be dispensed. Add 5 µl of sample and then 75 µl of the serum diluent to each well. Prepare the control wells by adding first 100 µl of the serum diluent to each well and then 5 µl of the positive control, 5 µl of the cut off control (in duplicate) and 5 µl of the negative control to the corresponding wells. If the assay is performed manually, shake the plate in a plate shaker (2 min) in order to achieve a homogenous 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 reagents and sample. Mix homogenously with the pipette and dispense 105 µl of each diluted sample to the wells.
6. Cover with a sealing sheet and incubate at 37±1°C 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 conjugate solution into each well.
9. Cover with a sealing sheet and incubate in incubator/water bath at 37±1°C 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 min protected from light.
13. Stop colour development by adding 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.
PERFORMANCES:

- **Sensitivity and Specificity:**
  75 serum/plasma samples were assayed with HELICOBACTER PYLORI ELISA IgA against another commercial available ELISA KIT.
  The results were as follows:

<table>
<thead>
<tr>
<th>Samples No.</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>94.44%</td>
<td>93.94%</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>CO</td>
<td>10</td>
<td>0.77</td>
</tr>
<tr>
<td>NC</td>
<td>10</td>
<td>7.30</td>
</tr>
<tr>
<td>PC</td>
<td>10</td>
<td>0.38</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>CO</td>
<td>10</td>
<td>0.77</td>
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<tr>
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</tbody>
</table>

C.V. Coefficient of variation

- **Cross Reactivity and Interferences:**
  6 samples known to be positive for other bacteria of the digestive tract (Salmonella) were assayed. 4 samples known to be positive for antinuclear antibodies 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.

**Symbols Used in Labels:**

- **IVD** - In vitro diagnostic medical device
- **Use by (expiration date)**
- **Store at x-yºC**
- **Contains sufficient for <X> test**
- **Batch code**
- **Catalogue number**
- **Consult instructions for use**
- **<X> wells**
BIBLIOGRAPHY:

For any question please contact: customerservice@vircell.com

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