**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 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 SERUM DILUENT:** 25 ml of serum dilution solution: a blue colourless phosphate buffer containing protein stabilizers and Proclin.

**STORAGE REQUIREMENTS:**

Store at 2-8ºC and check expiration date.

Materials required but not supplied:
- Precision micropipettes 5 and 100 µl.
- Eight channel micropipette 100 µl.
- ELISA plate washer.
- Thermostatted 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).

**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.

**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.

**FOR IN VITRO DIAGNOSTIC USE**

Manufacturer: **VIRCELL, S.L.** Pza. Dominguez Ortiz 1. Polígono Industrial Dos de Octubre; 18020 Santa Fe, GRANADA, SPAIN. Tel. +34.958.441264 Fax +34.958.510712 http://www.vircell.com
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 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 mucus. 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).

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 samples are to be 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 sera. 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.

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 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±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.

<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;0.5</td>
</tr>
<tr>
<td>CUT OFF CONTROL</td>
<td>&gt;0.55</td>
</tr>
<tr>
<td></td>
<td>&lt;1.5</td>
</tr>
</tbody>
</table>

INTERPRETATION OF RESULTS:

QUALITATIVE

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; 9</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 retested and/or a new sample obtained for confirmation.

Samples with indexes below 9 are considered as not having IgA specific antibodies against H. pylori.

Samples with indexes above 11 are considered as having IgA specific antibodies against H. pylori.

SEMIQUANTITATIVE

In order to estimate the relative concentration of specific anti-Helicobacter pylori IgA antibody present in the serum sample a semilogarithmic graph must be drawn. For example, the logarithmic of the U/mL of each control and the average of their corresponding O.D. could be represented. Then, double controls must be included in each test run. A straight line could be drawn between the two points that could allow to assign an approximate value of U/mL to the sample knowing its O.D.

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.

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http://www.vircell.com
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.-For IgA testing, human IgG sorbent must be used. Otherwise, false negative results may be obtained due to an excess of IgG antibodies.
7.-The lack of a rapid clear serologics response to eradication of bacterial infection makes the interpretation of serological results from follow-up of therapy be taken with precaution.
8.-When the sample result is expressed in U/ml, it should be taken into account that measured values above cut off do not correlate with the amount of antibodies present in the sample.

**PERFORMANCE**

**SENSITIVITY AND SPECIFICITY:**

75 serum samples were assayed with HELICOBACTER PYLORI ELISA IgA against another commercial available ELISA KIT.
The results were as follows:

<table>
<thead>
<tr>
<th>SAMPLE NR</th>
<th>SENSITIVITY</th>
<th>SPECIFICITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>75</td>
<td>94.44%</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>0.43</td>
</tr>
<tr>
<td>NC</td>
<td>10</td>
<td>6.15</td>
</tr>
<tr>
<td>CO</td>
<td>10</td>
<td>0.87</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>%</th>
<th>% C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>10</td>
<td>0.18</td>
</tr>
<tr>
<td>NC</td>
<td>10</td>
<td>5.30</td>
</tr>
<tr>
<td>CO</td>
<td>10</td>
<td>0.77</td>
</tr>
</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° C**
- **Contains sufficient for <X> test**
- **Batch code**
- **Catalogue number**
- **Consult instructions for use**
- **<X> wells**
- **<X> cells**

**SUMMARY OF THE ASSAY PROCEDURE**

- **Wash 5x (washing solution) 9**
- **100 µl conjugate**
- **Wash 5x (washing solution) 9**
- **100 µl de substrate**
- **50 µl stopping solution**

Read at 450/620 nm
LITERATURE:


For any question please contact:
customerservice@vircell.com

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