Instruction Manual

Helicobacter pylori IgG ELISA

Cat. No.: 07I-512002

Enzyme immunoassay
for the detection and quantitative determination
of human IgG antibodies against Helicobacter pylori
in serum and plasma

Storage: 4-8°C

February 2002

ICN Diagnostics

<table>
<thead>
<tr>
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1. Intended Use

The ICN Helicobacter pylori IgG Antibody ELISA test kit has been designed for the detection and the quantitative determination of specific IgG antibodies against Helicobacter pylori in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of ICN. This assay is intended for in-vitro diagnostic use only. Laboratory results can never be the only base of a medical report. The patient history and further tests have additionally to be taken into account.

2. General Information

Helicobacter pylori, a 2.5-3 µm long, twisted or helical gram-negative germ is responsible for 80-90 % of B-gastritis cases and is suspected to be a major cofactor for the development of gastric and duodenal ulcers. Up to now, Helicobacter pylori could be most reliably detected by culturing from mucous membrane biopsies; also a urease test was employed for identification. These detection methods are only successful for a relatively high germ count from the inoculate and require an identification directly after taking the biopsy. Recently it was shown that a detection of Helicobacter pylori by indirect immunofluorescence (IIF) should be superior to the above methods. The identification by IIF is also advantageous as it is not necessary to perform the determination directly after the biopsy; thus preparations can be sent to specialized laboratories.

The colonisation of the gastric and duodenal mucous membranes by Helicobacter pylori can also be detected serologically by the performance of an enzyme immunoassay (ELISA) or by Western Blot. Patients with confirmed exposition to Helicobacter pylori often show a positive serological result. Since antibodies persist for a longer time after a Helicobacter pylori infection, seropositive individuals are also found among symptom-free patients. The ratio of seropositive values rises with age. By the determination of immunoglobulins in ELISA and by the detection of IgA and IgG antibodies against specific proteins of Helicobacter pylori in the Western Blot, it should be possible to diagnose an acute infection with Helicobacter pylori, even if no germs can be found.

The detection of Helicobacter pylori is of remarkable interest for the therapy. Bismuth salts and antibiotics use to kill the germs, and these should complement or replace the usual therapy with antacidic and H2 blocking agents. Serological monitoring could thus also be employed to control the success of anti-microbial therapy.

3. Principle of the Test

The ICN Helicobacter pylori IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Helicobacter pylori antigen is bound on the surface of the microtiter strips. Diluted patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Helicobacter antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.
4. Limitations, Precautions and General Comments

- Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognised methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25°C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- No reagents from different kit lots have to be used, and they should not be mixed with one another.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

5. Reagents Provided

The kit contains sufficient reagents for 12 x 8 = 96 determinations. The strips and solutions have to be stored at 4-8 °C. The expiry date is mentioned on the labels.

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume / Quantity</th>
</tr>
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<tbody>
<tr>
<td>Helicobacter pylori antigen coated microtiter strips</td>
<td>12</td>
</tr>
<tr>
<td>Negative Control</td>
<td>2 mL</td>
</tr>
<tr>
<td>Cut-Off Standard</td>
<td>2 mL</td>
</tr>
<tr>
<td>Weak Positive Control</td>
<td>2 mL</td>
</tr>
<tr>
<td>Positive Control</td>
<td>2 mL</td>
</tr>
<tr>
<td>Enzyme Conjugate</td>
<td>12 mL</td>
</tr>
<tr>
<td>Substrate</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>12 mL</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>60 mL</td>
</tr>
<tr>
<td>Washing Buffer (10×)</td>
<td>60 mL</td>
</tr>
<tr>
<td>Plastic foils</td>
<td>2</td>
</tr>
<tr>
<td>Plastic bag</td>
<td>1</td>
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</tbody>
</table>
5.1. Mikrotiter Strips
12 strips with 8 breakable wells each, coated with a Helicobacter pylori antigen. Ready-to-use.

5.2. Negative Control
2 mL, human serum diluted with PBS, contains no IgG antibodies against Helicobacter. Addition of 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane. Ready-to-use.

5.3. Cut-Off Standard
2 mL human serum diluted with PBS, contains a low concentration of IgG antibodies against Helicobacter. Addition of 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane. Ready-to-use.

5.4. Weak Positive Control
2 mL, human serum diluted with PBS, contains a medium concentration of IgG antibodies against Helicobacter. Addition of 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane. Ready-to-use.

5.5. Positive Control
2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against Helicobacter. Addition of 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane. Ready-to-use.

5.6. Enzyme Conjugate
12 mL, anti-human-IgG-HRP, in protein-containing buffer solution. Addition of 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane. Ready-to-use.

5.7. Substrate
12 mL, TMB (tetramethylbenzidine). Ready-to-use.

5.8. Stop Solution
12 mL, 1 M sulfuric acid. Ready-to-use.

5.9. Sample Diluent
60 mL, PBS/BSA buffer. Addition of 0.1 % sodium azide. Ready-to-use.

5.10. Washing Buffer
60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

5.11. Plastic Foils
2 pieces to cover the microtiter strips during the incubation.

5.12. Plastic Bag
Resealable, for the dry storage of non-used strips.

6. Materials Required but not Provided
- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter plate reader (450 nm)
- Microtiter plate washer
- Reagent tubes for the serum dilution
- Bidistilled water
7. Specimen Collection and Handling

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (4-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:101 with ready-to-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

8. Assay Procedure

8.1. Preparation of Reagents

**Washing Solution:** dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- Standards and samples should be assayed in duplicate.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 4-8°C.

8.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.
2. Pipet 100 µL each of the *diluted* (1:101) samples and the *ready-to-use* standards and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.
4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9. Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.
9. Evaluation

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10%.

Example

<table>
<thead>
<tr>
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<th>OD Value</th>
<th>corrected OD</th>
<th>Mean OD Value</th>
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<tbody>
<tr>
<td>Substrate Blank</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>0.032 / 0.030</td>
<td>0.012 / 0.010</td>
<td>0.011</td>
</tr>
<tr>
<td>Cut-Off Standard</td>
<td>0.624 / 0.604</td>
<td>0.604 / 0.584</td>
<td>0.594</td>
</tr>
<tr>
<td>Weak Positive Control</td>
<td>0.874 / 0.902</td>
<td>0.854 / 0.882</td>
<td>0.868</td>
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<tr>
<td>Positive Control</td>
<td>2.083 / 2.061</td>
<td>2.063 / 2.041</td>
<td>2.052</td>
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</table>

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently no reference values which have to be found in each laboratory individually.

9.1. Qualitative Evaluation

The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result.

For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/- 20% around the value of the Cut-Off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run.

The positive control must show at least the double absorption compared with the cut-off standard.

9.2. Quantitative Evaluation

The ready-to-use standards and controls of the Helicobacter pylori antibody kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for controls and standards in units are printed on the labels of the vials.

For a quantitative evaluation the absorptions of the standards and controls are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

10. Assay Performance

The intra-assay coefficient of variation of the Helicobacter pylori IgG test kit was assessed by a ten-fold determination of a positive serum sample to less than 10%.
11. References

12. Ordering Information

<table>
<thead>
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