Product information

Instruction Manual

Helicobacter pylori IgM ELISA

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

Cat. No.: DEM-DEHEL03
Storage: 2-8°C
Regulatory: For in-vitro diagnostic use only
Product information

Contents

1. Intended Use...................................................................................................................................................3
2. General Information........................................................................................................................................3
3. Principle of the Test........................................................................................................................................3
4. Limitations, Precautions and General Comments ..........................................................................................4
5. Reagents Provided .........................................................................................................................................4
6. Materials Required but not Provided ...............................................................................................................5
7. Specimen Collection and Handling.................................................................................................................6
8. Assay Procedure.............................................................................................................................................6
9. Calculation of Results....................................................................................................................................7
10. Interpretation of Results................................................................................................................................7
11. Expected Values...........................................................................................................................................7
12. Performance / Assay Characteristics............................................................................................................8
13. References ...................................................................................................................................................8
1. Intended Use

The Demeditec Helicobacter pylori IgM Antibody ELISA Test Kit has been designed for the detection and the quantitative determination of specific IgM 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 Demeditec.

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 IgG, IgA and IgM 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 Demeditec Helicobacter pylori IgM antibody test kit is based on the principle of the enzyme immunoassay (EIA). Helicobacter 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 IgM 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-IgM 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 IgM 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 recognized 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, they should not be mixed among 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

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume / Qty.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helicobacter pylori antigen coated microtiter strips</td>
<td>12</td>
</tr>
<tr>
<td>Calibrator A (Negative Control)</td>
<td>2 mL</td>
</tr>
<tr>
<td>Calibrator B (Cut-Off Standard)</td>
<td>2 mL</td>
</tr>
<tr>
<td>Calibrator C (Weak Positive Control)</td>
<td>2 mL</td>
</tr>
<tr>
<td>Calibrator D (Positive Control)</td>
<td>2 mL</td>
</tr>
<tr>
<td>Enzyme Conjugate</td>
<td>15 mL</td>
</tr>
<tr>
<td>Substrate</td>
<td>15 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>15 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 bag</td>
<td>1</td>
</tr>
</tbody>
</table>

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. After the first opening the kit should be used within 3 months, the diluted wash buffer can be kept for 4 weeks at 2-8°C.
5.1. Microtiter Strips
12 strips with 8 breakable wells each, coated with a Helicobacter pylori antigen. Ready-to-use.

5.2. Calibrator A (Negative Control)
2 mL, protein solution diluted with PBS, contains no IgM antibodies against Helicobacter. Addition of 0.02% methylisothiazolone and 0.02% bromonitrodioxane. Ready-to-use.

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

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

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

5.6. Enzyme Conjugate
15 mL, anti-human-IgM-HRP (rabbit), in protein-containing buffer solution. Ready-to-use.

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

5.8. Stop Solution
15 mL, 0.5 M acidic solution. 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 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
- Re-usable black lid for covering (Available upon request at Demeditec Diagnostics GmbH)
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).

In order to avoid interference of rheumatoid factors, it is highly recommended that patient sera should be treated with RF absorbent (Cat.No. DE-MJS02). Alternatively, positive results can be confirmed in a second test run in the presence of RF adsorbent. Do not pre-treat the controls or standards with RF absorbent!

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 duplicates.
- 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 re-usable plate cover 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 re-usable plate cover 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 re-usable plate cover and incubate at room temperature for 20 minutes.
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. Calculation of Results

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log.

For the calculation of the standard curve apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read from the standards curve.

The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in “Assay Procedure” (chapter 8.) and reassayed.

**Typical Calibration Curve**  (Example. Do not use for calibration!)

<table>
<thead>
<tr>
<th>Standard</th>
<th>U/mL</th>
<th>Mean OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A  (Negative Control)</td>
<td>1</td>
<td>0.010</td>
</tr>
<tr>
<td>B  (Cut-Off Standard)</td>
<td>10</td>
<td>0.456</td>
</tr>
<tr>
<td>C  (Weak Positive Control)</td>
<td>25</td>
<td>0.821</td>
</tr>
<tr>
<td>D  (Positive Control)</td>
<td>150</td>
<td>2.176</td>
</tr>
</tbody>
</table>

10. Interpretation of Results

<table>
<thead>
<tr>
<th>U/mL</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 8</td>
<td>negative</td>
</tr>
<tr>
<td>8 - 12</td>
<td>equivocal</td>
</tr>
<tr>
<td>&gt; 12</td>
<td>positive</td>
</tr>
</tbody>
</table>

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

11. Expected Values

In an in-house study apparently healthy subjects showed the following results:

<table>
<thead>
<tr>
<th>Ig Isotype</th>
<th>n</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>IgM</td>
<td>72</td>
<td>1.4 %</td>
</tr>
</tbody>
</table>
12. Performance / Assay Characteristics

<table>
<thead>
<tr>
<th>Helicobacter pylori ELISA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay-Precision</td>
<td>8.1 %</td>
</tr>
<tr>
<td>Inter-Assay-Precision</td>
<td>11.3 %</td>
</tr>
<tr>
<td>Inter-Lot-Precision</td>
<td>4.4 – 12.7 %</td>
</tr>
<tr>
<td>Analytical Sensitivity</td>
<td>1.03 U/mL</td>
</tr>
<tr>
<td>Recovery</td>
<td>88 – 107 %</td>
</tr>
<tr>
<td>Linearity</td>
<td>84 – 122 %</td>
</tr>
<tr>
<td>Cross-Reactivity</td>
<td>No cross-reactivity to Yersinia enterocolitica</td>
</tr>
<tr>
<td>Interferences</td>
<td>No interferences to bilirubin up to 0.3 mg/mL, haemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL</td>
</tr>
<tr>
<td>Clinical Specificity</td>
<td>99 %</td>
</tr>
<tr>
<td>Clinical Sensitivity</td>
<td>85 %</td>
</tr>
</tbody>
</table>

13. References