Helicobacter pylori Antigen ELISA Kit

For the in vitro Determination of Helicobacter pylori in stool

Valid from 04.05.2011
1. INTENDED USE

This *Immundiagnostik* Assay is a sandwich ELISA for determination of *Helicobacter pylori* in stool. The test kit is for *in vitro* diagnostic use only.

2. INTRODUCTION

*Helicobacter pylori* is regarded as a causative factor for chronic B-gastritis, drug-unrelated ulcus duodeni, and as an etiologic stimulus of gastric MALT-lymphoma. Furthermore, it is suspected of being involved in the pathogenesis of stomach carcinoma.

The epidemiology of infection by *Helicobacter pylori* has been characterized in western industrial nations by a linear increase with age increasing and in developing countries by a large number of children and juveniles being affected.

The currently used methods for the diagnosis of *Helicobacter pylori* infection are very sensitive and highly specific, but all include either invasive sampling or require special technical devices.

We have developed an ELISA for the detection of *Helicobacter pylori* infection from faecal samples. This cost-efficient methodology provides a reliable result without the loss of sensitivity or specificity and does not require invasive sampling.

**Indications:**

- Detection of a *Helicobacter pylori* infection
- Monitoring the effect of a *Helicobacter pylori* treatment

3. PRINCIPLE OF THE TEST

A microtiter plate is coated with polyclonal antibodies specific for *Helicobacter pylori* which bind *Helicobacter pylori* from the patient sample in a first incubation step. After a washing step, the bound antigen, *Helicobacter pylori*, is incubated with a biotin-labeled antibody. After a further washing step, a peroxidase-labeled streptavidin is added. Then a chromogenic substrate (TMB) is added, followed by a stop solution. The color changes from blue to yellow. The intensity of the color is proportional to the amount of analyte (sample or control). The results are evaluated by comparison with a cut-off value.
4. MATERIAL SUPPLIED

<table>
<thead>
<tr>
<th>Catalogue No.</th>
<th>Content</th>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K 6920MTP</td>
<td>PLATE</td>
<td>One holder with precoated strips</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>K 6920WP</td>
<td>WASHBUF</td>
<td>ELISA wash buffer concentrate 10x</td>
<td>2 x 100 ml</td>
</tr>
<tr>
<td>K 6920PV</td>
<td>SAMPLEBUF</td>
<td>Sample dilution buffer; 2.5x</td>
<td>1 x 90 ml</td>
</tr>
<tr>
<td>K 6920VP</td>
<td>2.ABDIL</td>
<td>2.\textsuperscript{nd} Antibody dilution buffer, ready-to-use</td>
<td>1 x 20 ml</td>
</tr>
<tr>
<td>K 6920KO1</td>
<td>CTRL NEG</td>
<td>Control negative, lyophilized</td>
<td>4 vials</td>
</tr>
<tr>
<td>K 6920KO2</td>
<td>CTRL POS</td>
<td>Control positive, lyophilized</td>
<td>4 vials</td>
</tr>
<tr>
<td>K 6920A2</td>
<td>2.AB</td>
<td>2\textsuperscript{nd} Antibody (anti-Helicobacter pylori biotin-labeled)</td>
<td>1 x 200 μl</td>
</tr>
<tr>
<td>K 6920K</td>
<td>CONJ</td>
<td>Conjugate, peroxidase-labeled streptavidin</td>
<td>1 x 200 μl</td>
</tr>
<tr>
<td>K 6920TMB</td>
<td>SUB</td>
<td>TMB substrate (Tetramethylbenzidine), ready to use</td>
<td>1 x 15 ml</td>
</tr>
<tr>
<td>K 6920AC</td>
<td>STOP</td>
<td>ELISA stop solution, ready to use</td>
<td>1 x 15 ml</td>
</tr>
</tbody>
</table>

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled water (aqua bidest.)
- Precision pipettors calibrated and tips to deliver 10-1000 μl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 or 405 nm (reference wave length 620 or 690 nm)
6. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay.** The kit can be used up to 4 times within the expiry date stated on the label.

- Reagents with a volume less than 100 μl should be centrifuged before use to avoid loss of volume.

- The ELISA **WASHBUF** (wash buffer concentrate) should be diluted with aqua bidest. **1:10** before use (100 ml WASHBUF + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C before dilution of the buffer solutions. The **buffer concentrate** is stable at 2-8°C until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at 2-8°C for one month.

- The **SAMPLEBUF** (sample dilution buffer) should be diluted with aqua bidest. **1:2.5** before use (90 ml SAMPLEBUF + 135 ml aqua bidest), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C before dilution. The **buffer concentrate** is stable at 2-8°C until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at 2-8°C for three months.

- The **2.AB** (2. antibody) must be diluted **1:100** in **2.ABDIL** (2. antibody dilution buffer) (100 μl biotin-labelled **2.AB** + 9.9 ml **2.ABDIL**). The undiluted antibody is stable at 2-4°C up to the until expiry date given on the label. **Diluted antibody solution is not stable and cannot be stored.**

- The **CONJ** (conjugate, POD-labelled streptavidine) must be diluted **1:100** in wash buffer (100 μl CONJ + 9.9 ml wash buffer). The undiluted conjugate is stable at 2-4°C up to the until expiry date given on the label. **Diluted conjugate solution is not stable and cannot be stored.**

- The **CTRL NEG/CTRL POS** (controls, both negative and positive), must be reconstituted with **500 μl** bidest. water. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. **Reconstituted controls are not stable and cannot be stored.**
7. **SAMPLE PREPARATION**

**Faeces**

The test can be performed on either fresh or frozen stool samples. If the test cannot be performed within one day, the specimen should be stored at −20°C or colder.

Add a stool sample of **100 mg** to **1 ml** of the sample dilution buffer (SAMPLEBUF) and homogenize thoroughly on a Vortex-mixer. Centrifuge the suspension for 15 min at 3000 rpm.

*Immundiagnostik* recommends for sample preparation the use of Roche Diagnostics / Mannheim sample preparation tubes, article No. 10745804332.

8. **ASSAY PROCEDURE**

**Procedural notes**

- Do not interchange different lot numbers of any kit component within the same assay.
- Quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. *Immundiagnostik* AG can therefore not be held responsible for any damage resulting from wrong use.
- The assay should always be performed according the enclosed manual.
**Test procedure**

Carry out the tests in duplicate. Cover the microtiter plate during the different incubation steps. Bring all reagents samples to room temperature before use and mix well. Avoid direct sunlight during all incubation steps. Wash the precoated microtiter strips 5 x with 250 μl diluted wash buffer before use. Afterwards dry the microtiter plate on absorbent paper.

1. Add 100 μl of **CTRL POS/CTRL NEG/SAMPLEBUF/SAMPLE** (control positive, control negative, sample dilution buffer (blank) and samples (stool suspension)) in duplicate into respective well.

2. Cover and incubate for **1 hour** shaking on a horizontal mixer at room temperature. Alternatively, incubate **overnight** at 2-8° C without shaking.

3. Aspirate and wash the wells 7 x with 250 μl diluted wash buffer.

4. Add 100 μl diluted **2.AB** (2nd antibody, biotin-labelled) into each well.

5. Cover and incubate for **1 hour**, shaking on a horizontal mixer at room temperature.

6. Aspirate and wash the wells 5 x with 250 μl diluted wash buffer.

7. Add 100 μl diluted **CONJ** (Conjugate, Peroxidase-labelled) into each well.

8. Cover and incubate for **1 hour**, shaking on a horizontal mixer at room temperature.

9. Aspirate and wash the wells 5 x with 250 μl diluted wash buffer.

10. Add 100 μl **SUB** (substrate) into each well.

11. Incubate for **5-15 minutes** at room temperature in the dark.

12. Add 50 μl **STOP** (stop solution) into each well and mix shortly.

13. Determine absorption with an ELISA reader at **450 nm** against 620 nm as reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the positive control exceeds the measurement range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as reference.
9. RESULTS

Calculation of the values

Blank absorbance must be < 0.150. If the absorbance of the blank is higher, the analysis must be repeated.

Test result

Cut off-value = 0.150 at 450/620 nm and 0.190 at 450 nm

Samples with absorbance more than 0.020 (measurement at 450/620 nm) or 0.025 (measurement at 450 nm) above the cut off-value are considered as positive.

Samples with an absorbance 0.020 (0.025) below or above the cut off-value are regarded as border line samples and must be re-analyzed.

If the value from the repeated measurement is again in the border line range, a new sample should be analyzed.

If the value from the repeated measurement is again in the range above the cut off-value, the result is considered as positive.

Samples with absorbance more than 0.020 (0.025) under the cut off-value are classified as negative.

10. QUALITY CONTROL

The test is correct when the absorption of the negative control at 450 nm is less than 0.190 and at 450/620 nm less than 0.150, and the positive control value at 450 nm greater than 0.54 and at 450/620 nm greater than 0.50. If the observed values differ from the expected, the test must be repeated.

Immundiagnostik AG recommends the use of commercial available controls for internal quality control.

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods.
11. PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

*Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the test*

<table>
<thead>
<tr>
<th></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical sensitivity</td>
<td>97,7</td>
</tr>
<tr>
<td>Clinical specificity</td>
<td>96,3</td>
</tr>
<tr>
<td>Positive predictive value (PPV)</td>
<td>98,8</td>
</tr>
<tr>
<td>Negative predictive value (NPV)</td>
<td>92,9</td>
</tr>
</tbody>
</table>

Samples, n = 113; H. pylori positive, n = 86; H. pylori negative n = 27

12. PRECAUTIONS

- The test kit is for *in vitro* diagnostic use only
- Quality control guidelines should be observed.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay.
- Reagents should not be used beyond the expiry date shown on the kit label.
- Substrate solution should remain colorless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for in vitro diagnostic use only.
- Guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

Used symbols:

- Temperature limitation
- Catalogue Number
- In Vitro Diagnostic Medical Device
- Contains sufficient for <n> tests
- Manufacturer
- Use by
- Lot number