Helicobacter pylori Antigen ELISA

Catalog Number: HEL31-K01

Enzyme immunoassay for the determination of Helicobacter pylori antigen in fecal specimens

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v. 1.0

INTENDED USE

The Eagle Biosciences Helicobacter pylori Antigen ELISA Assay Kit is used for the qualitative detection of Helicobacter pylori antigen in fecal specimen.

Helicobacter pylori (H. pylori) is a gram-negative spiral shaped microaerophilic bacterium that can colonize the human stomach.

In the stomach H. pylori protects itself from destruction by gastric acid through incorporation into the gastric mucosa and through cleavage of urea to ammonia and carbon dioxide. The ammonia neutralizes the gastric acid in the contiguity of the germ. This reaction is catalyzed by the bacterial enzyme urease that can also be used as diagnostic proof (Helicobacter-Urease-test).

H. pylori infections are often associated with stomach disorders characterized by increased production of gastric acid, for example type B gastritis, about 75% of Ulcus ventriculi and almost all cases of Ulcus duodeni.

Therefore the screening of H.pylori infection is an important part in the diagnostics of stomach diseases. Chronic infections with H. pylori are furthermore an accepted high risk factor for the development of gastric malignomas and MALT lymphoma. Therefore, in 1944 the WHO has dedicated H. pylori into group I of the defined carcinogens.

Immunological tests on the basis of specific anti-H. pylori antibodies enable a noninvasive and direct detection of H. pylori antigens from stool specimen and other matrices and may be used for diagnostic screening and for therapeutic surveillance.

PRINCIPLE OF THE TEST

Helicobacter pylori Antigen is an enzymometric one step sandwich immunoassay for the qualitative determination of Helicobacter pylori antigen based on monoclonal antibodies.

Samples or calibrators and horseradish peroxidase (HRP) conjugated anti-H. pylori antibodies are dispensed simultaneously into the wells of the microplate coated with monoclonal anti-H. pylori antibodies. After 60 minutes at 22-25°C non-bound material is removed by a washing step.

HRP converts the subsequently added colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) into a blue product. The enzyme reaction is terminated by sulphuric acid dispensed into the
wells after 15 min incubation at 22-25°C turning the solution from blue to yellow.

The optical density (OD) of the solution read at 450 nm is directly proportional to the specifically bound amount of *Helicobacter pylori* antigen. For optimal results a reference filter (620 nm wavelength) should be used. Results are interpreted as positive or negative considering a cut-off value.

### SAMPLE PREPARATION

**Specimen collection and storage**

The stool samples should be stored at 2-8°C immediately after collection and processed within 48 hours. Longer storage is possible at -20°C. Repeated freezing and thawing of samples should be avoided.

Avoid contact with water and urine!

**Sample preparation**

Quickly thaw frozen samples; warm samples to room temperature and mix well.

Pipette 1000 µl of sample diluent into a clean tube.

Using a disposable stirring rod transfer about 100 mg (diameter about 2-3 mm) of faeces if solid or pipette 100 µl if liquid into the tube and suspend thoroughly.

If necessary, sediment floating particles by a centrifugation step.

### TEST COMPONENTS FOR 96 DETERMINATIONS

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Microtiter plate, 12 breakable strips per 8 wells coated with monoclonal antibodies to <em>Helicobacter pylori</em> (mice)</td>
<td>1</td>
<td>vacuum sealed with desiccant</td>
</tr>
<tr>
<td>B</td>
<td>Wash buffer, 25 fold concentrated solution, sufficient for 1000 ml solution</td>
<td>40 ml</td>
<td>concentrate capped white</td>
</tr>
<tr>
<td>C</td>
<td>Sample diluent</td>
<td>100 ml</td>
<td>ready for use capped white</td>
</tr>
<tr>
<td>D</td>
<td>Conjugate monoclonal anti-<em>Helicobacter pylori</em> antibodies (mice) HRP conjugated</td>
<td>8 ml</td>
<td>ready for use capped white</td>
</tr>
<tr>
<td>E</td>
<td>Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide</td>
<td>2 bottles x 8 ml</td>
<td>ready for use capped blue</td>
</tr>
<tr>
<td>F</td>
<td>Stop solution 0.25 sulfuric acid</td>
<td>8 ml</td>
<td>ready for use capped yellow</td>
</tr>
<tr>
<td>1 - 3</td>
<td>Calibrators H. pylori antigen positive sample, inactivated</td>
<td>3 x 1 ml</td>
<td>ready for use capped blue, white and red</td>
</tr>
</tbody>
</table>

**Materials required but not provided**

- micropipettes
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water
- samples tubes for preparation of faecal specimens
- disposable stirring rods
- vortex
- centrifuge (optional)

### Size and storage

The Eagle Biosciences *Helicobacter pylori* Antigen ELISA Assay Kit has been designed for 96 determinations.

The expiry date of each component is reported on its respective label, that of the complete kit on the box labels.

Upon receipt, all components of the *Helicobacter pylori* Antigen have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.
Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 25 times (1 + 24) with distilled or deionized water. For example, dilute 10 ml of the concentrate with 240 ml of distilled water. The wash solution prepared is stable at 2 - 8 °C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

**ASSAY PROCEDURE**

- Dilute samples with sample diluent (C) 1 + 10 (w/v), e.g. 100 mg (100 µl) stool + 1 ml sample diluent (C)
- Avoid any time shift during pipetting of reagents and samples.

<table>
<thead>
<tr>
<th>wells</th>
<th>OD (a)</th>
<th>OD (b)</th>
<th>OD (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator 1</td>
<td>0.070</td>
<td>0.090</td>
<td>0.080</td>
</tr>
<tr>
<td>Calibrator 2</td>
<td>0.596</td>
<td>0.552</td>
<td>0.574</td>
</tr>
<tr>
<td>Calibrator 3</td>
<td>3.125</td>
<td>3.378</td>
<td>3.252</td>
</tr>
</tbody>
</table>

Cut-off determination

Cut-off OD = (OD CAL2 + OD CAL1) / 2

Samples with absorbances lower than or equal to the cut-off value are considered negative, samples with absorbances higher than the cut-off value are considered positive for Helicobacter pylori antigen.

<table>
<thead>
<tr>
<th>Helicobacter pylori</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; Cut-off</td>
<td>≥ Cut-off</td>
</tr>
</tbody>
</table>

**Example of typical assay results**

Sample 1 | 1.218 | 1.186 | 1.202 positive
Sample 2 | 0.148 | 0.156 | 0.152 negative

It is recommended that each laboratory establishes its own normal and pathological reference ranges as usually done for other diagnostic parameters too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

**Test validity**

The test run is valid if:

- the mean OD of calibrator 1 is ≤ 0.20
- the mean OD of calibrator 3 is ≥ 1.20

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times...
and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

**Limitations of the procedure**

There is no correlation between measured absorbance and seriousness of the infection.

Cross contamination of reagents and samples can produce false positive results. Incorrect dilutions, not sufficiently homogenized samples or solid particles after centrifugation of the suspension can cause false negative as well as false positive results.

A negative test result in the Helicobacter pylori Antigen ELISA does not exclude an infection:

The overall interpretation of the ELISA results should always consider the microbiological examination as well as clinical findings.

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**CHARACTERISTIC ASSAY DATA**

**Lower detection limit**

The lower detection limit of the Helicobacter pylori Antigen ELISA has been determined by titration of purified H. pylori antigen.

The lower detection limit is 4 - 8 ng/ml.

**Sensitivity and specificity**

Specificity and sensitivity of the Helicobacter pylori Antigen ELISA were determined investigating 34 stool samples from patient with suspected H. pylori infection in comparison to another commercially available ELISA. The overall agreement was 97 %. One sample that was negative in the competitor ELISA was found clearly positive in the Helicobacter pylori Antigen ELISA.
**Helicobacter Antigen ELISA Assay Kit**

### ASSAY SCHEME

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>Bring all reagents to room temperature (RT, 20-25°C)</strong></td>
</tr>
<tr>
<td>2</td>
<td><strong>Dispense Calibrators (1,2,3)</strong></td>
</tr>
<tr>
<td></td>
<td>1+10 (w/v) prediluted fecal specimens</td>
</tr>
<tr>
<td></td>
<td>1 drop (50 µl)</td>
</tr>
<tr>
<td></td>
<td>100 µl</td>
</tr>
<tr>
<td>3</td>
<td><strong>Dispense conjugate (D)</strong></td>
</tr>
<tr>
<td></td>
<td>1 drop (50 µl)</td>
</tr>
<tr>
<td>4</td>
<td><strong>Cover plate and incubate</strong></td>
</tr>
<tr>
<td></td>
<td>60 minutes, RT (20-25 °C)</td>
</tr>
<tr>
<td>5</td>
<td><strong>Wash</strong></td>
</tr>
<tr>
<td></td>
<td>Decant, 5 x 300 µl wash solution (made of B)</td>
</tr>
<tr>
<td>6</td>
<td><strong>Dispense substrate (E)</strong></td>
</tr>
<tr>
<td></td>
<td>2 drops (100 µl)</td>
</tr>
<tr>
<td>7</td>
<td><strong>Incubate protected from light</strong></td>
</tr>
<tr>
<td></td>
<td>15 minutes, RT (20-25 °C)</td>
</tr>
<tr>
<td>8</td>
<td><strong>Dispense stop solution (F)</strong></td>
</tr>
<tr>
<td></td>
<td>1 drop (50 µl)</td>
</tr>
<tr>
<td>9</td>
<td><strong>Read at 450 nm against 620 (690) nm within 30 min</strong></td>
</tr>
</tbody>
</table>
SAFETY PRECAUTIONS

- **This Eagle Biosciences Helicobacter Antigen ELISA Assay Kit is for research use only.** Follow the working instructions carefully. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots (except: diluent, washing buffer, substrate and stop solution).
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Since the Helicobacter Antigen ELISA Assay Kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

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*For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.*