Enzyme immunoassays for the diagnosis of *Helicobacter pylori* infection

**ELISA** and **IMMUNOBLOT** kits are optimized and validated for detection of IgA, IgG and IgM antibodies in human serum and plasma.

Diagnostic medium for rapid urease activity detection in bioptic sample.
INTRODUCTION

*Helicobacter pylori* belongs in the genus Helicobacter. Morphologically, it is a Gram-negative, microaerophilic bacterium. It is found as a pathogen in patients with infection of the gastric mucosa, particularly in the area of pyloric antrum and duodenum. It is a causative agent of B-type chronic gastritis, which is linked to the development of gastric ulcers. In this case, *H. pylori* is detected in 100% of individuals. *H. pylori* infection is often associated with dyspepsia. Active chronic gastritis can further develop in the atrophy of stomach lining and increase the risk of gastric carcinoma.

The factors of *H. pylori* pathogenicity are based on both the morphologic structure of the bacteria cells (helix-shaped curved rod, flagella) and its ability to produce extracellular enzymes and cytotoxins (e.g. urease, catalase, protease, VacA and CagA).

Bacterial strains can be pathogenic or facultatively pathogenic. Their virulence depends on the qualitative and quantitative representation of the above mentioned factors. Their pathogenesis is also influenced by a host response. Resistant strains are isolated mainly from unsuccessfully treated patients.

DIAGNOSIS OF INFECTION

Methods for *H. pylori* detection can be invasive and non-invasive. The most commonly used invasive methods are the rapid urease test and histological and cytological examination of bioptic sample of the gastric mucosa. Non-invasive techniques involve a breath test and serological methods (detection of IgM, IgA and IgG antibodies in the serum). Non-invasive tests are suitable for observation of treatment efficiency as well as for determination of infection or reinfection status. Eradication of the microbial agent is followed by a decrease of the antibody level. Tests involving highly specific and sensitive techniques of molecular biology (PCR) are only performed at specialized laboratories.

DIAGNOSTIC IMPORTANCE OF ANTIBODY CLASSES

**IgM**: The level of IgM antibodies increases in the acute stage of the disease. Nevertheless, they might not be produced by all patients.

**IgA**: IgA antibodies are produced not only in the acute stage of the disease, but also in the case of chronic infection of gastric mucosa, along with IgG antibodies. Their increase is also described in patients with a risk of gastric carcinoma.

**IgG**: IgG antibodies indicate contact with *H. pylori*; however, they do not provide any evidence of infection activity. Seroconversion occurs approximately 2 months after primary infection.

DETERMINATION OF UREASE ACTIVITY IN BIOPTIC SAMPLE OF THE GASTRIC MUCOSA

Due to the urease activity of most of *H. pylori* strains, urea undergoes hydrolysis in diagnostic medium. This process leads to the production of ammonia and therefore the pH of the sample is being increased. Hence the colour of pH indicator is changed from yellow to red.
ELISA

TEST PRINCIPLE

Sandwich ELISA

- **HRP**
- **Anti-Hu IgG**
- **IgG**
- **Ag**

- **HRP**
- **Anti-Hu IgM**
- **IgM**
- **Ag**

- **HRP**
- **Anti-Hu IgA**
- **IgA**
- **Ag**

Microtiter plate

**SUMMARY PROTOCOL**

<table>
<thead>
<tr>
<th>Step</th>
<th>Test steps</th>
</tr>
</thead>
</table>
| 1    | Dilute samples  
• serum/plasma 1:101 (10 μl + 1 ml) |
| 2    | Pipette controls and diluted samples 100 μl  
• Blank = empty well |
| 3    | Incubate 30 minutes at 37 °C |
| 4    | Aspirate and wash the wells 5 times |
| 5    | Add 100 μl Conjugate  
• Including blank |
| 6    | Incubate 30 minutes at 37 °C |
| 7    | Aspirate and wash the wells 5 times |
| 8    | Add 100 μl Substrate (TMB-Complete)  
• Including blank |
| 9    | Incubate 15 minutes at 37 °C |
| 10   | Add 100 μl Stopping solution  
• Including blank |
| 11   | Read colour intensity at 450 nm |

**ANTIGENS**

Clinically significant *Helicobacter pylori* strain with high content of CagA (120 kDa) and VacA (87 kDa) proteins.

**CLINICAL APPLICATION**

- Screening test for the detection of specific IgA, IgG and IgM antibodies in human serum or plasma
- Checking of therapy results using the semiquantitative determination
- Disease stage diagnosis

**USER COMFORT**

- Ready-to-use components
- Colour-coded components
- Interchangeable components
- Breakable colour-coded microplate strips
- CUT-OFF and calibrators included
- Semiquantitative evaluation of results (Index of Positivity) or quantitative evaluation of results (U/ml)

**ADVANTAGES**

- High diagnostic specificity and sensitivity
- High reproducibility
- High dynamics of antibody response
- Identical assay procedure
- Short total assay time 1.5 hour
- Quantitative evaluation available
- Ready for automation
- Customer support

**TEST CHARACTERISTICS**

<table>
<thead>
<tr>
<th>ELISA</th>
<th>Diagnostic Sensitivity</th>
<th>Diagnostic Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA Helicobacter MONO IgA</td>
<td>98.7%</td>
<td>98.8%</td>
</tr>
<tr>
<td>EIA Helicobacter MONO IgG</td>
<td>98.9%</td>
<td>98.8%</td>
</tr>
<tr>
<td>EIA Helicobacter MONO IgM</td>
<td>97.5%</td>
<td>97.4%</td>
</tr>
</tbody>
</table>
IMMUNOBLOT

TEST PRINCIPLE
Recombinant antigens *H. pylori* are transferred to a nitrocellulose membrane

SUMMARY PROTOCOL

<table>
<thead>
<tr>
<th>Step</th>
<th>Test steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pipette Universal solution 2 ml</td>
</tr>
</tbody>
</table>
| 2 | Strips soaking 10 min. at room temperature  
  • Shaker |
| 3 | Aspirate |
| 4 | Dilute samples  
  • serum/plasma 1:51 (30 μl + 1.5 ml) |
| 5 | Pipette Controls and diluted samples 1.5 ml |
| 6 | Incubate 30 min. at room temperature  
  • Shaker |
| 7 | Aspirate samples and wash strips with 1.5 ml  
  of Universal solution 3-times for 5 min.  
  • Shaker |
| 8 | Pipette Conjugate 1.5 ml |
| 9 | Incubate 30 min. at room temperature  
  • Shaker |
| 10 | Aspirate Conjugate and wash strips with 1.5 ml  
  of Universal solution 3-times for 5 min.  
  • Shaker |
| 11 | Pipette Substrate solution (BCIP/NBT) 1.5 ml |
| 12 | Incubate 15 min. at room temperature  
  • Shaker |
| 13 | Aspirate Substrate solution and wash strips with 2 ml of distilled water 2-times for 5 min.  
  • Shaker |
| 14 | Sticking and evaluation of strips |

CLINICAL APPLICATION

- Confirmatory method for the ELISA test
- Detailed determination of the presence of antibodies against specific antigens of *H. pylori*

USER COMFORT

- Ready-to-use components
- Colour-coded components
- Interchangeability components
- Positive and Negative controls
- Control line on the strip
- Possibility of software evaluation

ADVANTAGES

- Simple interpretation and reproducibility of results
- High diagnostic specificity and sensitivity
- Customer support
- Ready for automation

TEST CHARACTERISTICS

<table>
<thead>
<tr>
<th>Patogen</th>
<th>Diagnostic Sensitivity</th>
<th>Diagnostic Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLOT-LINE Helicobacter IgA</td>
<td>95.2%</td>
<td>93.9%</td>
</tr>
<tr>
<td>BLOT-LINE Helicobacter IgG</td>
<td>96.9%</td>
<td>96.3%</td>
</tr>
</tbody>
</table>
**ANTIGENS**

- **CagA, p120** – Cytotoxin associated gene A, highly specific, virulence factor
- **VacA, p87** – Vacuolating cytotoxin A, highly specific, virulence factor
- **UreA, p29** – Light subunit of urease, specific, virulence factor
- **NAP** – Neutrophil-activating protein, virulence factor, potential biomarker of gastritis
- **HpaA** – Helicobacter pylori adhesin A, surface lipoprotein, potential biomarker of gastritis and gastric ulcer
- **HcpC** – Helicobacter cystein-rich protein, virulence factor
- **GroEL** – Chaperonin, heat shock protein (Hsp 60), virulence factor, is considered a marker of chronic infection

**CLINICAL DATA**

Correlation of the BLOT-LINE Helicobacter results with EIA and Immunoblot kits

<table>
<thead>
<tr>
<th>BLOT-LINE Helicobacter</th>
<th>IgG</th>
<th>No. of Tests</th>
<th>IgA</th>
<th>No. of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA (TestLine)</td>
<td>92,2</td>
<td>90</td>
<td>84,4</td>
<td>90</td>
</tr>
<tr>
<td>EIA (Chorus)</td>
<td>94,1</td>
<td>34</td>
<td>84,9</td>
<td>33</td>
</tr>
<tr>
<td>BLOT (competition 1)</td>
<td>97,8</td>
<td>15</td>
<td>87,5</td>
<td>15</td>
</tr>
<tr>
<td>BLOT (competition 2)</td>
<td>100,0</td>
<td>15</td>
<td>80,0</td>
<td>15</td>
</tr>
</tbody>
</table>

**UREASE ACTIVITY DETECTION**

**WORKING SCHEDULE AND EVALUATION**

- Sample
- Incubation at room temperature for 1 hour
- Incubation at room temperature for 3 hours
ORDERING INFORMATION

ELISA

<table>
<thead>
<tr>
<th>Cat. No</th>
<th>Product</th>
<th>No. of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMA096</td>
<td>EIA Helicobacter MONO IgA</td>
<td>96</td>
</tr>
<tr>
<td>HMG096</td>
<td>EIA Helicobacter MONO IgG</td>
<td>96</td>
</tr>
<tr>
<td>HMM096</td>
<td>EIA Helicobacter MONO IgM</td>
<td>96</td>
</tr>
<tr>
<td>SK-HMA096</td>
<td>SmartEIA Helicobacter MONO IgA</td>
<td>96</td>
</tr>
<tr>
<td>SK-HMG096</td>
<td>SmartEIA Helicobacter MONO IgG</td>
<td>96</td>
</tr>
<tr>
<td>SK-HMM096</td>
<td>SmartEIA Helicobacter MONO IgM</td>
<td>96</td>
</tr>
</tbody>
</table>

SmartEIA kits are designed for automated processing using the Agility® analyser.

IMMUNOBLOT

<table>
<thead>
<tr>
<th>Cat. No</th>
<th>Product</th>
<th>No. of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>HpAL20</td>
<td>BLOT-LINE Helicobacter IgA</td>
<td>20</td>
</tr>
<tr>
<td>HpGL20</td>
<td>BLOT-LINE Helicobacter IgG</td>
<td>20</td>
</tr>
<tr>
<td>Swlm03</td>
<td>Immunoblot Software</td>
<td>1 pc</td>
</tr>
</tbody>
</table>

UREASE ACTIVITY DETECTION

<table>
<thead>
<tr>
<th>Cat. No</th>
<th>Product</th>
<th>No. of Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ut0050</td>
<td>UREASAtest 50</td>
<td>50</td>
</tr>
<tr>
<td>Utb100</td>
<td>UREASAtest bulk</td>
<td>100</td>
</tr>
</tbody>
</table>

CONTACT

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E-mail: sales@testlinecd.com
www.testlinecd.com

Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.