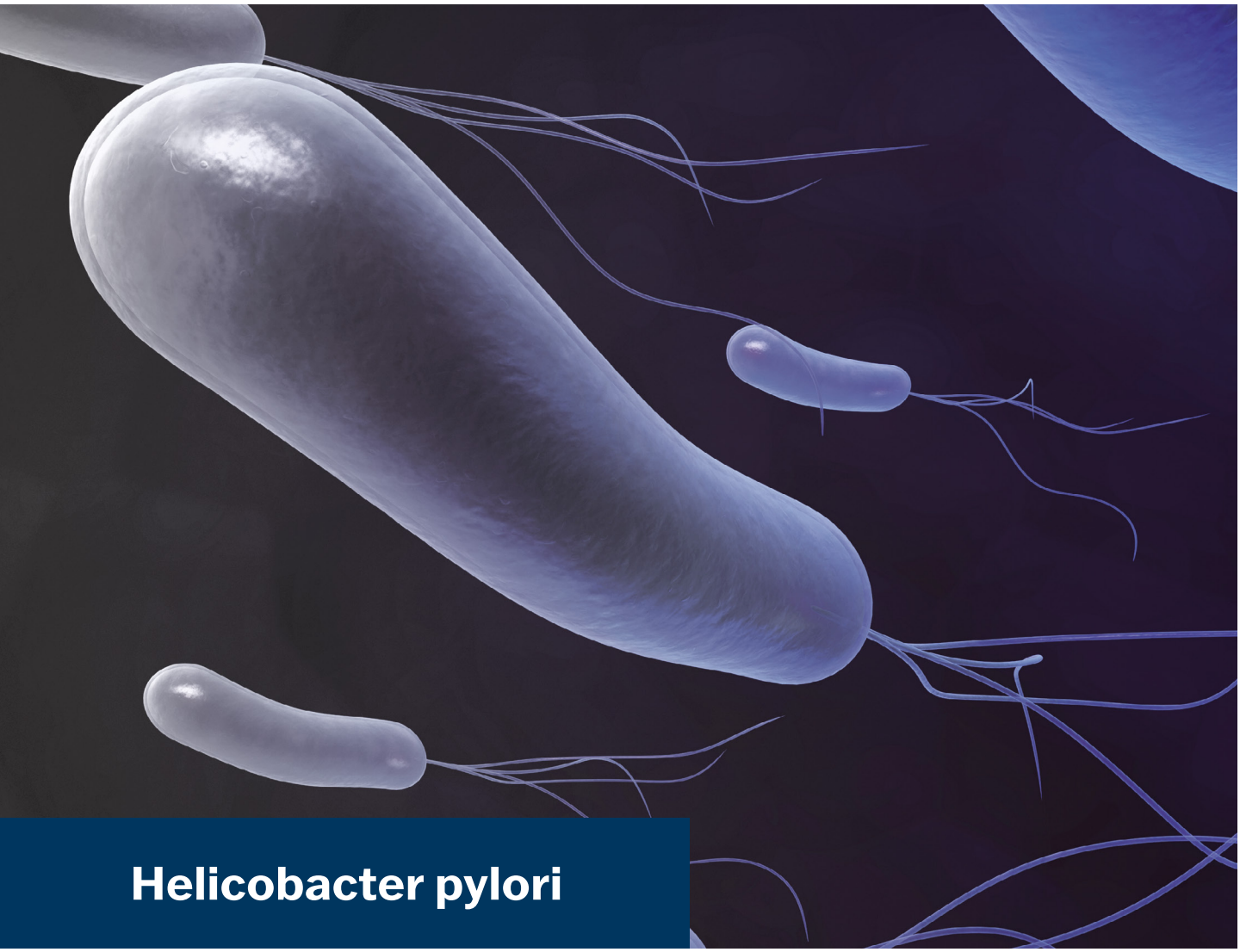


Helicobacter pylori

Enzyme immunoassays for the diagnostics of Helicobacter pylori infection

ELISA, **IMMUNOBLOT**, and **MICROBLOT-ARRAY** 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

CE IVD

Diagnostic kits are intended for professional use in the laboratory.

BG TestLine®

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.

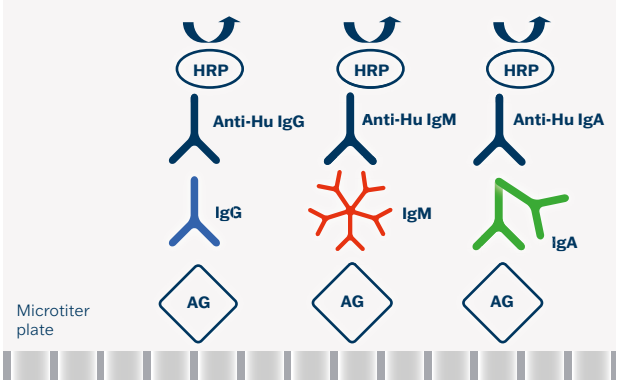
Determination of Urease Activity in Bioptic Sample of the Gastric Mucosa

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

The assays are based on a sandwich type of ELISA method.



Protocol Summary

Step		Test steps
	1.	Dilution of samples – serum/plasma 1:101 (10 µl + 1 ml)
	2.	Pipette Controls and diluted samples 100 µl – Including blank
	3.	Incubate 30 min. at 37 °C
	4.	Aspirate and wash the wells 5 times
	5.	Add Conjugate 100 µl – Including blank
	6.	Incubate 30 min. at 37 °C
	7.	Aspirate and wash the wells 5 times
	8.	Add 100 µl Substrate (TMB-Complete) – Including blank
	9.	Incubate 15 min. 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

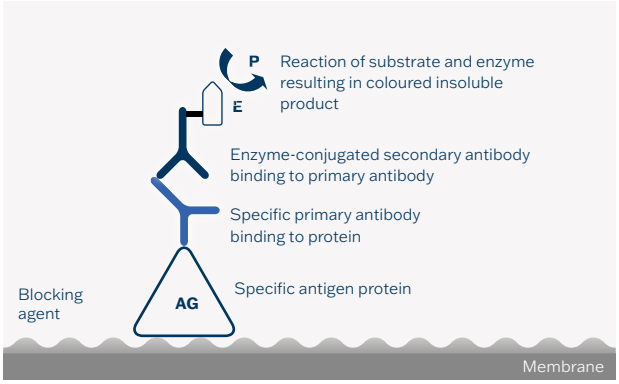
Test Characteristics

ELISA	Diagnostic Sensitivity	Diagnostic Specificity
EIA Helicobacter MONO IgA	98.7%	98.8%
EIA Helicobacter MONO IgG	98.9%	98.8%
EIA Helicobacter MONO IgM	97.5%	97.4%

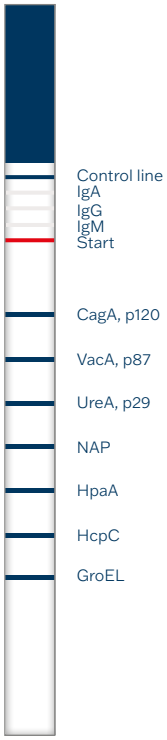
IMMUNOBLOT

Test Principle

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



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

Protocol Summary

Step		Test steps
	1.	Pipette Universal solution 2 ml
	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

Pathogen	Diagnostic Sensitivity	Diagnostic Specificity
BLOT-LINE Helicobacter IgA	95.2%	93.9%
BLOT-LINE Helicobacter IgG	96.9%	96.3%

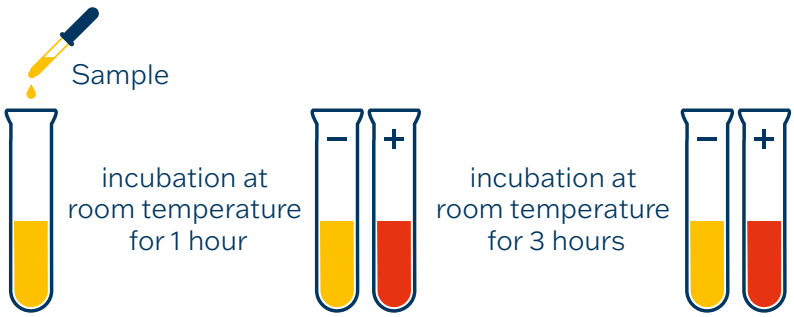
Clinical Data

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

BLOT-LINE Helicobacter	Conformity in %			
	IgG	No. of Tests	IgA	No. of Tests
EIA (TestLine)	92.2	90	84.4	90
EIA (Chorus)	94.1	34	84.9	33
BLOT (competition 1)	97.8	15	87.5	15
BLOT (competition 2)	100.0	15	80.0	15

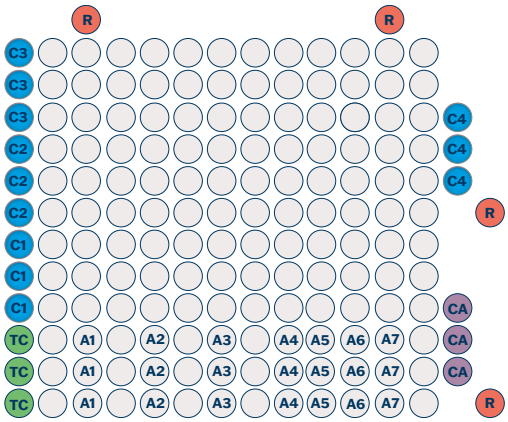
UREASE ACTIVITY DETECTION

Working schedule and evaluation



MICROBLOT-ARRAY

Distribution of Antigens and Control Spots



Description of antigens

A1 – CagA
A2 – VacA
A3 – UreA
A4 – NAP
A5 – HpaA
A6 – HcpC
A7 – GroEL

Description of control spots

R – Reference
TC – Test control
CA – Conjugate control IgA
CG – Conjugate control IgG
C1 – Calibration 1
C2 – Calibration 2
C3 – Calibration 3
C4 – Calibration 4

Protocol Summary

Step		Test steps
	1.	Pipette Universal solution 150 µl
	2.	Strips soaking 10 min. at room temperature
	3.	Aspirate
	4.	Dilute samples – serum/plasma 1:51 (10 µl + 500 µl)
	5.	Pipette Controls and diluted samples 100 µl
	6.	Incubate 30 min. at room temperature
	7.	Quick wash with Universal Solution*
	8.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	9.	Pipette Conjugate 100 µl
	10.	Incubate 30 min. at room temperature
	11.	Quick wash with Universal Solution*
	12.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	13.	Pipette Substrate solution (BCIP/NBT) 100 µl
	14.	Incubate 15 min. at room temperature
	15.	Quick wash using the distilled water *
	16.	Aspirate Substrate solution and wash strips with 200 µl of distilled water 2-times for 5 min.
	17.	Dry and evaluate strips

* if using a washer, fill the wells to the brim and aspirate immediately after filling the last well

User Comfort

- Low sample consumption
- Antigens spotted in triplicate – minimizing statistical variation
- Possibility of automatic assay processing and results evaluation
- Parallel testing of multiple markers simultaneously
- High sensitivity and specificity

Test Characteristics

Pathogen	Diagnostic Sensitivity	Diagnostic Specificity
Microblot-Array Helicobacter IgA	96.5%	99.1%
Microblot-Array Helicobacter IgG	97.4%	99.0%

Ordering Information

ELISA

Cat. No	Product	No. of Tests
HMA096	EIA Helicobacter MONO IgA	96
HMG096	EIA Helicobacter MONO IgG	96
HMM096	EIA Helicobacter MONO IgM	96
SK-HMA096	SmartEIA Helicobacter MONO IgA	96
SK-HMG096	SmartEIA Helicobacter MONO IgG	96
SK-HMM096	SmartEIA Helicobacter MONO IgM	96

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

IMMUNOBLOT

Cat. No	Product	No. of Tests
HpAL20	BLOT-LINE Helicobacter IgA	20
HpGL20	BLOT-LINE Helicobacter IgG	20
SwIm03	Immunoblot Software	1 pc

MICROBLOT-ARRAY

Cat. No	Product	No. of Tests
HpAMA48	Microblot-Array Helicobacter IgA	48
HpGMA48	Microblot-Array Helicobacter IgG	48

UREASE ACTIVITY DETECTION

Cat. No	Product	No. of Tests
Ut0050	UREASAtest 50	50
Utb100	UREASAtest bulk	100

TestLine Clinical Diagnostics Ltd.
Krizikova 68, 612 00 Brno, Czech Republic
+420 549 121 203
sales@testlinecd.com
www.testlinecd.com

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

V10/2022