TestLine®

Instruction for use

EIA Helicobacter MONO IgG

REF HMG096

Σ 96

Kit for professional use

IVD CE

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1 Intended Use

Enzyme immunoassay for the detection of IgG antibodies *to Helicobacter pylori* in human serum or plasma.

2 Introduction

Helicobacter pylori belongs to the genus *Helicobacter.* It represents a key pathogenic factor in 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 may result in development of gastric ulcers or in the atrophy of stomach lining. This increases the risk of gastric carcinoma. *H. pylori* infection is often associated with dyspepsia, epigastric pain, peptic ulcer disease or MALT lymphoma.

There are several invasive and non-invasive methods for *H. pylori* detection. Commonly used invasive tests of biopsy sample include a rapid urease test and histological and cytological examination. Non-invasive techniques involve a breath test and serological methods (detection of IgA, IgG and IgM antibodies in the serum). Non-invasive tests are suitable for monitoring of treatment efficiency as well as for screening for infection or reinfection. Eradication of the microbial agent is followed by a decrease of the antibody level.

IgA antibodies are produced not only in the acute stage of the disease but also in chronic infection of gastric mucosa (along with IgG antibodies). Increased level of IgA antibodies was also described in patients with a risk of gastric carcinoma.

Presence of IgG antibodies indicates contact with *H. pylori*. However, it does not provide any evidence of infection activity. Seroconversion occurs approximately 2 months after primary infection.

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

3 Test Principle

The kit is intended for detection of specific IgG antibodies in a sample by means of a sandwich type of the EIA method (i.e. a solid phase coated with specific antigen – antibody from the analysed sample – labelled antibody). The labelled antibody (conjugate) is an animal immunoglobulin fraction to human IgG conjugated with horseradish peroxidase. Peroxidase activity is determined in the test by a substrate containing TMB. Positivity is indicated when blue colour appears; after stopping solution has been added, blue changes to yellow. The yellow colour intensity is measured by a photometer at 450 nm, and it is proportional to the concentration of specific IgG antibodies in the sample.

Antigen Used

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

4 Materials Provided

MICROPLATE	**Microtitre Plate** coated with antigen, 12 x 8 wells in bag with desiccant	1 pc
CONTROL - CAL1	**Negative Control (Calibrator 1) 10 U/ml** Solution containing no specific human antibodies, ready to use	1 × 2 ml
CUTOFF CAL2	**CUT-OFF (Calibrator 2) 20 U/ml** Solution containing specific human antibodies in cut-off concentration, ready to use	1 × 3 ml
CONTROL + CAL3	**Positive Control (Calibrator 3) 120 U/ml** Solution containing specific human antibodies, ready to use	1 × 2 ml
CAL4	**Calibrator 4 (640 U/ml)** Solution containing specific human antibodies, ready to use	1 × 2 ml
CONJUGATE	**Conjugate** Solution containing peroxidase labelled animal immunoglobulin to human IgG, ready to use	1 × 15 ml
DILUENT 2	**Sample Diluent 2** Buffer with protein stabilisers, ready to use	1 × 105 ml
SUBSTRATE 2	**TMB-Complete 2** Chromogenic substrate solution containing TMB/H_2O_2, ready to use	1 × 15 ml
WASH 20x	**Wash Solution** 20× concentrated buffer	1 × 75 ml
STOP	**Stop Solution** Acid solution, ready to use	1 × 15 ml
	Instructions for use	1 pc

5 Other Material Required for Manual Test Performance

Single and multichannel pipettes

Disposable tips

Microplate washer

Timer

Incubator (37°C)

Microplate reader

6 Storage and Stability

Store the kit at +2°C to +8°C. Do not freeze. If the kit is stored as described, the labelled expiration date is valid. The expiration date is indicated on the package. The opened kit should be used within three months.

Sample Preparation and Storage

The following human body liquids can be used for testing: serum and citrate plasma. Anticoagulants in the plasma (except for citrate) as well as bacterially contaminated, haemolytic or chylous samples can affect the test results.

Samples can be stored at +2°C to +8°C for one week. For a longer period, store samples at -20°C. Diluted samples should be used as soon as possible.

7 Preparation of Reagents

Dilute the Wash Solution 1:20 (1 part of solution and 19 parts of distilled water); e.g. 75 ml of the concentrated Wash Solution + 1425 ml of distilled water.

Salt crystals might develop in the bottle with the concentrated Wash Solution. Prior to use, it is necessary to dissolve the crystals by warming the bottle in a water bath. The diluted Wash Solution is stable at +2°C to +8°C for one week.

The Controls and the Calibrators are supplied ready to use, do not dilute further!

The Conjugate is supplied ready to use, do not dilute further!

TMB-Complete is a one-component chromogenic substrate solution ready to use, do not dilute further!

Interchangeability of reagents

The Sample Diluent, TMB-Complete and the Avidity Solution are interchangeable in EIA kits of TestLine Clinical Diagnostics s.r.o., provided they have the identical numeric marking (e.g. Sample Diluent 2, Sample Diluent 3, etc.). The Stop Solution and the Wash Solution are universal in all kits.

8 Preparation of Samples

Mix gently the Sample Diluent prior to use.

Dilution of sera and plasma samples

Dilute well mixed samples 1:101 with the Sample Diluent:

E.g.: 10 µl of sample + 1 ml of the Sample Diluent

Mix well.

9 Assay Procedure

Allow all reagents to come to room temperature and mix well. If you do not use a whole microplate, return unnecessary strips into the bag with desiccant. Seal the bag tightly and store at +2°C to +8°C. Keep dry!

1. Dispense the controls (calibrators) and the diluted samples according to the working schedule.

Semiquantitative evaluation in Index of Positivity (IP)

- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 1 well.
- Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into the other wells.

Quantitative evaluation in Units U/ml

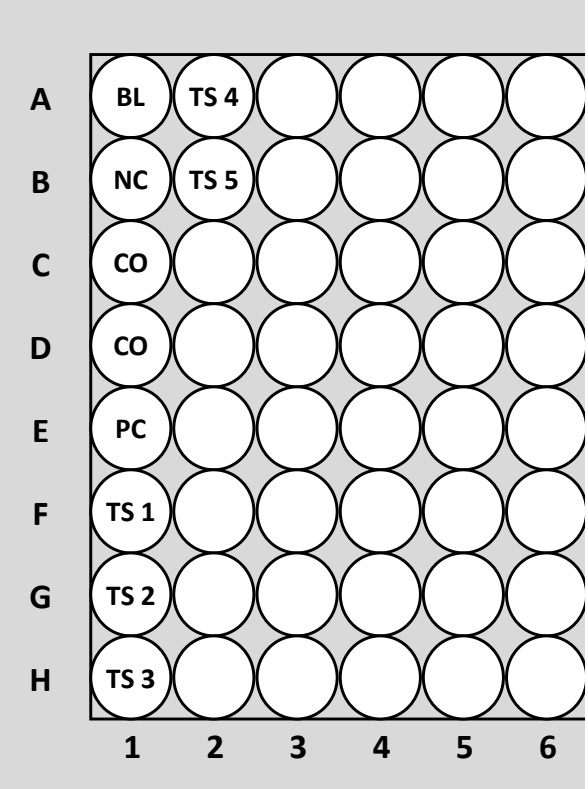
- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 2 wells.
- Pipette 100 µl of the Calibrator 4 into 2 wells.
- Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into the other wells.

2. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
3. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
4. Pipette 100 µl of the Conjugate into all wells except A1 well.
5. Cover the microplate with the lid and incubate at 37°C for 30 minutes.

6. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
7. Pipette 100 µl of TMB-Complete into all wells. Avoid contamination – see Chapter Procedural Notes.
8. Cover the microplate with the lid and incubate at 37°C for 15 minutes. Keep out of light.
9. Stop the reaction by adding 100 µl of the Stop Solution in the same order and intervals as the substrate was added.
10. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction.

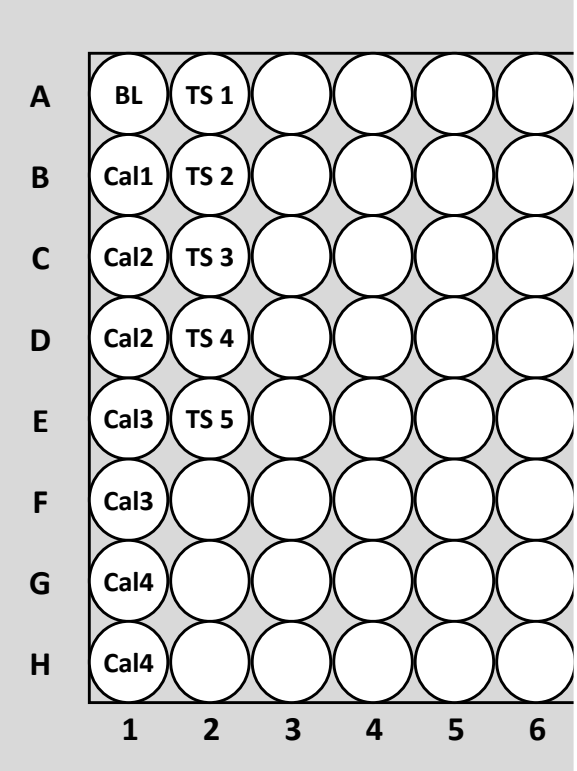
10 Working Schedule

Semiquantitative evaluation
Index of Positivity (IP)



BL	Blank (empty well)	
NC	100 µl	CONTROL - CAL1
CO	100 µl	CUTOFF CAL2
PC	100 µl	CONTROL + CAL3
TS 1-x	100 µl	diluted tested sample

Quantitative evaluation
Units U/ml



BL	Blank (empty well)	
Cal1	100 µl	CONTROL - CAL1
Cal2	100 µl	CUTOFF CAL2
Cal3	100 µl	CONTROL + CAL3
Cal4	100 µl	CAL4
TS 1-x	100 µl	diluted tested sample

11 Quality Control

The test is valid if:

The absorbance of blank is lower than 0.150.

BLANK < 0.150

The absorbance of the Negative Control (Calibrator 1) is lower than half of the mean absorbance of CUT–OFF (Calibrator 2).

CONTROL	-	CAL1	< 0.5 ×	CUTOFF	CAL2

The mean absorbance of CUT-OFF (Calibrator 2) is within a range of 0.200 – 0.800.

0.200 <	CUTOFF	CAL2	< 0.800

The absorbance of the Positive Control (Calibrator 3) is 1.5-fold higher than the mean absorbance of CUT–OFF (Calibrator 2).

CONTROL	+	CAL3	> 1.5 ×	CUTOFF	CAL2

The absorbance of the Calibrator 4 is higher than the absorbance of the Positive Control (Calibrator 3).

CAL4	>	CONTROL	+	CAL3

12 Results Interpretation

Calculation of Index of Positivity (IP)

Divide the absorbance of a tested sample by the mean absorbance of CUT-OFF measured in the same test run:

$$IP = \frac{\text{Absorbance of sample}}{\text{Mean absorbance of CUT-OFF}}$$

Interpretation of the test results is described in the table (Table 1).

Table 1 Interpretation of test results

Index of Positivity (IP)	Evaluation
lower than 0.9	negative
0.9 to 1.1	borderline
higher than 1.1	positive

Examination of borderline samples, i.e. samples with Index of Positivity from 0.9 to 1.1, should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

Quantitative evaluation in Units (U/ml)

Construct a calibration curve by plotting the concentration (X) of the calibrators in U/ml against the corresponding absorbance (Y). Construct the calibration curve by single point cross connection. Read the values of antibody level (U/ml) in samples from the calibration curve. Interpretation of the quantitative test results is described in the table (Table 2).

Table 2 Quantitative interpretation in Units (U/ml)

Antibody level (U/ml)	Evaluation
lower than 18	negative
18 to 22	borderline
higher than 22	positive

Examination of borderline samples should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

Serological finding can be interpreted only in the context of results of other laboratory tests and patient clinical picture.

13 Test Performance

13.1 Specificity and Sensitivity

Diagnostic specificity was determined in the panel of negative sera. Diagnostic sensitivity was determined in the panel of positive sera. The number of sera tested and the results obtained are described in the table (Table 3).

13.2 Reproducibility

Reference control samples were tested in a statistically significant number of replicates, either in one or several analyzes. Acquired data was used for Intra assay and Precision within the laboratory. The obtained results are described in the table (Table 3).

13.3 Analytical Sensitivity – maximum threshold sensitivity

The analytical sensitivity is the maximum binary dilution of CUT-OFF-like or low positive sample, possibly international recognized standards, giving absorbance significantly different from the background. The value is expressed in units of U/ml. This value is a minimum limit of detection and quantification. The obtained results are described in the table (Table 3).

13.4 Intra-homogeneity

The intra-homogeneity is expressed as the amount of agreement among 100 replicates of CUT OFF-like or low positive serum in one analyze. The value is expressed as a coefficient of variation. The obtained results are described in the table (Table 3).

13.5 Measuring range of the kits

The measuring range of each kit lies between the values of the lowest and the highest calibrators.

Table 3 Test Performance

Parameter	Value
Specificity (n 88)	98.86%
Sensitivity (n 90)	98.89%
Intra-assay	3.28%
Within-laboratory precision	6.35%
Analytical sensitivity limit	3.42 U/ml
Analytical sensitivity limit – index of positivity (IP)	0.17
Intra-homogeneity	7.62%

13.6 Interference

Two samples (one negative plasma pool and one positive plasma pool) were spiked with potentially interfering endogenous substances. Results of interference testing are shown in the table (Table 4).

Table 4 Interference Results

Interfering substance	The result was not affected up to concentration:
Bilirubin	0.4 mg/ml
Triacylglycerols	20 mg/ml
Hemoglobin	5 mg/ml
Biotin	3500 ng/ml

13.7 Cross-reactivity

The assay was evaluated for potential cross-reactivity using samples positive for selected pathogens and factors. Results of testing are shown in the table (Table 5).

Table 5 Results of Cross-Reacting Pathogens or Factors

Category	n	Positive Result
Borrelia spp.	21	0
Treponema pallidum	14	0
CCP	11	0
RF	8	0
Yersinia spp.	7	1
EBV	4	0
Total	**65**	**1**

14 Safety Precautions

The kit is intended for in vitro diagnostic use only.

The sera used for controls were tested and found to be negative for HIV 1 and HIV 2, HBsAg, HCV, TPHA. In spite of this fact, they still need to be handled as potentially infectious materials.

Some reagents contain sodium azide, which is a toxic compound. Avoid contact with skin.

The Stop Solution contains diluted acid solution. Avoid contact with eyes and skin.

It is necessary to observe the local safety rules and regulations.

First aid

In case of contact with eyes, flush with copious amount of water and seek medical assistance. In case of contact with skin and clothing, remove all the contaminated clothes. Wash the skin with soap and plenty of running water. In case of contact with solutions containing plasma or clinical samples, disinfect the skin. In case of accidental ingestion, flush the mouth with drinking water and seek medical assistance.

Remnants disposal

All the materials used for performing the test must be treated as potentially infectious due to the contact with biological materials. Therefore they need to be disposed together with biological waste.

Expired kit disposal

Disassemble the kit and dispose the components as biological material. Discard the packaging material as required by local regulations.

15 Procedural Notes

In order to obtain reliable results, it is necessary to **strictly follow the Instructions for Use**. Always use clean preferably disposable tips and glassware.

Microtitre Plate – in order to prevent water condensation on the surface of the microplate, always allow the bag with the microplate to warm up to room temperature before opening.

Wash Solution – use high quality distilled water for preparing the working strength Wash Solution.

Washing procedure – keep to the prescribed number of wash cycles and fill the wells to the upper edge. The soak time (i.e. interval between two different wash cycles during which the wells stay filled up with the Wash Solution) should be approx. 30-60 seconds.

TMB-Complete – the vessel used for multichannel pipetting should not be used for other reagents. Do not return the surplus TMB-Complete from the pipetting vessel into the vial.

Non-reproducible results might be caused by improper methodology as following:

- insufficient mixing of reagents and samples before use
- improper replacement of vial caps
- using the same tip for pipetting different reagents
- reagent exposure to excessive temperature; bacterial or chemical contamination
- insufficient washing or filling of the wells (the wells should be filled to the upper edge), improper aspiration of Wash Solution remnants
- contamination of the well edges with Conjugate or samples
- using reagents from different kit lots
- contact of reagents with oxidants, heavy metals and their salts

The kit might be used for sequential examinations. When preparing working strength solutions, use only the amount of reagents needed for the analysis.

The kit might be used in all types of automatic EIA analysers.

If necessary, TestLine Clinical Diagnostics s.r.o. can offer a certified modification of the Instructions for Use for the specific type of analyser.

The producer cannot guarantee that the kit will function properly if the assay procedure instructions are not strictly adhered to.

16 References

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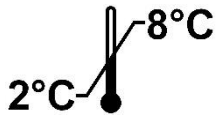
8. Wang YK, Kuo FC, Liu CJ, Wu MC, Shih HY, Wang SS, Wu JY, Kuo CH, Huang YK, Wu DC. Diagnosis of Helicobacter pylori infection: Current options and developments. *World J Gastroenterol*. 2015, 21(40), 11221-11235.

17 IFU Symbols

	Temperature limitation
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	Expiry date
	Lot number
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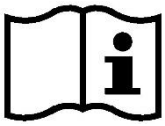	Consult instructions
	Catalogue number
	Number of tests
	In vitro diagnostic medical device

Notes

Summary of EIA Helicobacter MONO IgG Protocol

Step No.	Symbol	Test steps
1		Dilute samples serum/plasma 1:101 (10 µl + 1 ml)
2		Pipette Controls (Calibrators) and diluted samples – 100 µl Blank = empty well
3		Incubate at 37°C for 30 min
4		Aspirate and wash the wells 5×
5		Pipette Conjugate – 100 µl Blank = empty well
6		Incubate at 37°C for 30 min
7		Aspirate and wash the wells 5×
8		Pipette Substrate (TMB-Complete) – 100 µl Including blank
9		Incubate at 37°C for 15 min
10		Pipette Stop Solution – 100 µl Including blank
11		Read colour intensity at 450 nm