Instruction for use

A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUALITATIVE DETERMINATION OF IgG ANTIBODIES TO *HELICOBACTER PYLORI* IN HUMAN SERUM OR PLASMA

Helicobacter pylori IgG EIA

REF **K119**

For 96 determinations

IVD

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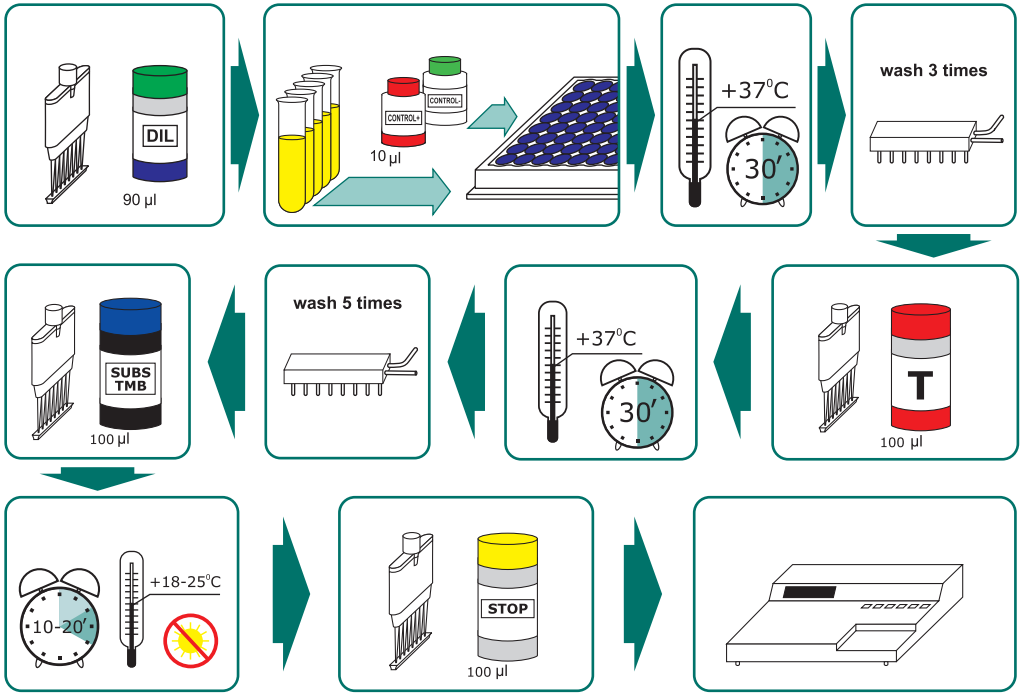
Test procedure



K119

CONTENT

Instruction for use

A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUALITATIVE DETERMINATION OF IgG ANTIBODIES TO *HELICOBACTER PYLORI* IN HUMAN SERUM OR PLASMA

1. INTENDED USE

A solid-phase enzyme immunoassay for the qualitative determination of IgG antibodies to *Helicobacter pylori* in serum or plasma.

For possibility of use with other sample types, please, refer to Application Notes (on request). The kit contains reagents sufficient for 96 determinations and allows to analyze 46 unknown samples in duplicates.

2. SUMMARY AND EXPLANATION

H. pylori is detectable in nearly 100% of adult patients with duodenal ulcer, about 80% of patients with gastric ulcer, more than 90% of gastric cancer patients and more than 90% of patients with active chronic gastritis. An association between *H. pylori* and gastric cancer is confirmed. In developing countries, where prevalence of *H. pylori* infection is high (ca. 70-80% of population for Eastern Europe and Asia), most children become infected by the age of 10, and gastric cancer rates are very high. In the USA and other developed countries, standards of hygiene and the increasing socioeconomic status of the population have reduced the incidence of infection, and in parallel, the rates of peptic ulcers and gastric cancer have declined. There is an excellent correlation between the clinical presentation of gastritis, the presence of *H. pylori* in the stomach and elevated serum *H. pylori* IgG antibody. Moreover, eradication of H. pylori from a patient leads to a recovery from gastritis and a significant reduction in duodenal ulcer reccurence. *H. pylori*-specific IgG antibody also falls significantly after successful antibacterial therapy.

H. pylori strains are classified into two broad groups - those that express both VacA (vacuolising toxin) and CagA (cytotoxin-associated gene) (type I) and those that produce neither (type II). Type I strains are predominant in patients with ulcers and cancer, while type II strains are mostly associated with asymptomatic infections. 80-100% of patients with duodenal ulcer disease produce CagA antibodies against a 128 kd antigen compared with 60-63% of *H. pylori*-infected persons with gastritis only, indicating that serologic responses to the 128 kd protein are more prevalent among *H. pylori*-infected persons with duodenal ulcers than infected persons without peptic ulceration. In *H. pylori*-infected patients who develop gastric cancer or duodenal ulcer, serum IgG against CagA are found in 94% and 80-100% of patients, resp., thus indicating that detection of antibodies to CagA is a useful marker for diagnosis of gastric cancer and duodenal ulcer. For gastritis, prevalence of IgG-antibodies to *H. pylori* is lower – ca. 60-63%.

Thus, determination of IgG antibodies to *H. pylori* CagA is a reliable and non-invasive method of diagnostics of *H. pylori* infection and monitoring of its antibacterial treatment.

3. PRINCIPLE OF THE TEST

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated with CagA protein of *Helicobacter pylori*. Antibodies from the specimen bind coated CagA protein of *Helicobacter pylori* on the microwell surface. Unbound material is removed by washing procedure. Second peroxidase-labeled antibodies directed towards human IgG, are then added into the microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Positivity index (PI, %) is calculated by the formula (see Calculations).Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

4. WARNINGS AND PRECAUTIONS

4.1. For professional use only.

4.2. This kit is intended for in vitro diagnostic use only.

4.3. INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are not present in the reagents of this kit. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

4.4. Avoid contact with stop solution containing 5.0 % H_2SO_4. It may cause skin irritation and burns.

4.5. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.

4.6. Do not use the kit beyond the expiration date.

4.7. All indicated volumes have to be performed according to the protocol. Optimal test results are obtained only when using calibrated pipettes and microplate readers.

4.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

4.9. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

4.10. Do not mix reagents from different lots.

4.11. Replace caps on reagents immediately. Do not swap caps.

4.12. Do not pipette reagents by mouth.

4.13. Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.

4.14. Material Safety Data Sheet for this product is available upon request directly from XEMA Co., Ltd.

4.15. Material Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

5. KIT COMPONENTS

5.1. Contents of the Kit

	Symbol	Description		Qty	Units	Colour code	Stability of opened/ diluted components
1	SORB MTP	*Helicobacter pylori IgG EIA strips, 8x12 wells*	Polystyrene microwells coated with CagA protein of *Helicobacter pylori*.	1	pcs		until exp.date
2	CONTROL - CONTROL +	Control sera (0.5 ml and 0.2 ml, resp.)	Dilution of preselected human serum, with high content of IgG antibodies to *Helicobacter pylori* with preservative - 0.01% Bronidox L, 0.01% 2-Methyl-4-isothiazolin-3-one-hydrochloride, colourless.	2	pcs	colourless; red	2 months
3	CONJ HRP	Conjugate 14 ml	Aqueous solution of murine antibodies to human IgG coupled with horseradish peroxidase diluted on phosphate buffered solution preservative - 0,01% Bronidox L, 0,01% 2-Methyl-4-isothiazolin-3-one-hydrochloride and red dye.	1	pcs	red	until exp.date
4	S011Z	EIA Sample buffer 14 ml	Phosphate buffered saline with casein from bovine milk and detergent (Tween-20), contains 0.1% phenol as preservative; contains blue dye.	1	pcs	blue	until exp.date
5	SUBS TMB	Substrate solution, 14 ml	Ready-to-use single-component tetramethylbenzidine (TMB) solution.	1	pcs	colourless	until exp.date
6	BUF WASH 26X	Washing solution concentrate 26X, 22 ml	Aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative.	1	pcs	colourless	concentrate - until exp.date Diluted was-hing solution - 45 days at 2-8 °C or 15 days at RT
7	STOP	Stop solution, 14 ml	5.0% vol/vol solution of sulphuric acid.	1	pcs	colourless	until exp.date
8	N003	Plate sealing tape		2	pcs		N/A
9	K119IE	Instruction Helicobacter pylori IgG EIA		1	pcs		N/A
10	K119Q	QC data sheet Helicobacter pylori IgG EIA		1	pcs		N/A

5.2 Equipment and material required but not provided:
- distilled or deionized water;
- automatic or semiautomatic multichannel micropipettes, 100-250 µl, is useful but not essential;
- calibrated micropipettes with variable volume, range volume 10-250 µl;
- dry thermostat for 37 °C ±2 °C;
- calibrated microplate photometer with 450 nm wavelength and OD measuring range 0-3.0.

5.3. Storage and stability of the Kit.
Store the whole kit at 2 to 8 °C upon receipt until the expiration date.
After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

6. SPECIMEN COLLECTION AND STORAGE.

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.
Specimens may be stored for up to 48 hours at 2...8 °C before testing. For a longer storage, the specimens should be frozen at -20 °C or lower. Repeated freezing/thawing should be avoided.

7. TEST PROCEDURE

7.1. Reagent Preparation:
- All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18 to +25 °C) before use.
- All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
- It is recommended to spin down shortly the vials with calibrators on low speed centrifuge.
- Prepare washing solution from the concentrate BUF WASH 26X by 26 dilution in distilled water.

7.2. Procedural Note.
It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

7.3. Assay flowchart.
Please, refer to the inside back cover.

7.4. Assay procedure.

1	Put the desired number of microstrips into the frame; allocate 4 wells for control samples CONTROL - CONTROL + (3 and 1 wells resp.) and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.
2	Pipet 90 µl of EIA Sample buffer into each well.
3	Pipet 10 µl of control samples CONTROL - CONTROL + and unknown samples into the wells. Cover the wells by plate adhesive tape (included into the κit).
4	Incubate 30 minutes at 37 °C.
5	Prepare washing solution by 26X dilution of washing solution concentrate BUF WASH 26X with distilled water. Minimal quantity of washing solution should be 250 µl per well. Wash strips 3 times.
6	Dispense 100 µl of CONJ HRP into the wells. Cover the wells by plate adhesive tape.
7	Incubate 30 minutes at 37°C.
8	Wash the strips 5 times.
9	Dispense 100 µl of SUBS TMB into the wells.
10	Incubate 10-20 minutes at 18...25 °C.
11	Dispense 100 µl of STOP into the wells.
12	Measure OD (optical density) at 450 nm.
13	Set photometer blank on air.

8. QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

For the assay to be valid, the following requirements should be met:

1. OD450 for CONTROL+ should be nlt 0.6 AU.
2. OD450 for CONTROL- should not be more than 0.15 AU for all replicates.

9. CALCULATION OF RESULTS

1. Calculate the mean absorbance values (OD450) for CONTROL- in triplicates and each pair of samples.
2. Calculate the cut-off value: (mean OD450 for CONTROL-) + 0.3.
3. Calculate Positivity Index (PI) for each sample:

PI = mean OD450(sample) / Cut-off.

10. EXPECTED VALUES

If PI value is greater than 1.1, the result is POSITIVE.

If PI value is less than 0.9, the result is NEGATIVE.

If PI value is between 0.9 and 1.1, the result is EQUIVOCAL. Such samples should be retested. If the result is equivocal again, a new sample should be obtained 2-4 weeks later and tested again. If the result remains equivocal, the sample should be considered negative. Therapeutic consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutic measures. NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

Use of PI allows to carry out semi-quantitative comparative determination of antibodies to H. pylori in serial samples taken with 2-4 weeks intervals, as PI is directly proportional to the level of IgG antibodies to *H. pylori* in the range 1.1-7.0.

If PI is above 7.0, it is recommended to dilute the specimen 10-fold before analysis and, when calculating PI, to multiply the PI obtained for the diluted sample, to 10.

11. PERFORMANCE CHARACTERISTICS

11.1 Analytical specificity.

Specificity of the test was evaluated on 158 negative serum specimens. Based on these data, specificity of the test is 98%.

11.2 Analytical sensitivity.

Sensitivity of the test was evaluated using internal panel of 42 samples confirmed positive. All samples were found positive - so, sensitivity of the test is 100%.

11.3 Precision.

Intra-assay precision for two different lots (CV1, CV2) is shown below:

Serum, no	Replicates	PI, mean	CV1, %	CV2, %
1	32	3.4	2.9	2.8
2	32	9.1	4.8	5.0

Inter-assay precision is shown below:

Serum, no	No of runs	PI, mean	CV1, %
1	8	3.1	3.9
2	8	9.0	6.2