BIOHIT HealthCare

Innovating for Health

GastroPanel®
Helicobacter pylori

ELISA kit for the detection of human IgG antibodies to *H. pylori* in EDTA plasma as part of GastroPanel

INSTRUCTIONS FOR USE

GastroPanel®
Product Family
606 400

REF 606 040 IVD CE

For *in vitro* diagnostic use

Store at 2-8 °C upon receipt

Biohit Oyj Laippatie 1, FI-00880 Helsinki, Finland
Tel. +358 9 773 861, info@biohit.fi, www.biohithealthcare.com

EXPLANATION OF THE SYMBOLS USED IN LABELS

	English
IVD	For *in vitro* diagnostic use
REF	Catalogue Number
LOT	Batch code
	Use by
	Consult instructions for use
+2°C +8°C	Storage limitation Store at +2…+8 °C
Σ 96	96 determinations
	Do not reuse
CE	CE Mark
WASH 10x	Washing Buffer Concentrate (10x)
S DILBUF	Sample Diluent Buffer
CAL	Calibrator
CONTROL	Control
CONJ	Conjugate
SUBS	Substrate
STOP	Stop solution

INSTRUCTIONS FOR USE **English**

Note! Other languages available at www.biohithealthcare.com

GastroPanel® *Helicobacter pylori* **Cat. No. 606 040**

1. INTRODUCTION TO GASTROPANEL®

GastroPanel® is the first-line diagnostic test for *Helicobacter pylori* (*Hp*) infection (5-80% of the world population), for the examination of all patients with dyspepsia (20-40% of the Western population), as well as for the screening of atrophic gastritis (AG) with related risks, such as stomach and esophageal cancer (1-3). Atrophic gastritis also enhances the risk of malabsorption of vitamin B12, iron, magnesium, zinc, calcium and some medicines.
GastroPanel consists of key stomach-specific biomarkers representing the key regulators of normal stomach physiology. These four biomarkers include pepsinogen I (PGI), pepsinogen II (PGII), amidated gastrin-17 (G-17), and *Hp* antibodies, designed to give information on both the structure and function of the stomach mucosa (1-6). Most importantly, this panel gives accurate estimates of the capacity of the corpus and antrum mucosa to secrete gastric acid and G-17, respectively, as well as of important gastric pathologies, like inflammation, grade and topography of atrophic gastritis (7-9), which may represent increased risk of gastric cancer (1).
Normal plasma levels of all four biomarkers indicate that the stomach mucosa has normal structure and function, whereas abnormal levels are signs of a non-healthy stomach, reflecting disturbances in the feedback mechanisms between the acid output of the corpus, PGs and G-17. For G-17 assessment, there are two options; G-17 basal (G-17b) values, and G-17 stimulated (G-17s) values, the latter being particularly important in distinguishing between functional disturbance of the antrum (G-17s normal) and AG in the antrum (G-17s does not increase in AG) (10,11).

Being the first non-invasive diagnostic test for stomach mucosal health, GastroPanel is unique in that the results are interpreted by a software application (GastroSoft) (http://www.GastroPanel.com), specifically designed for this purpose. GastroPanel results are classified into one of five possible diagnostic categories related to stomach morphology: 1) normal mucosa, 2) superficial or non-atrophic (*Hp*) gastritis, 3) AG in the corpus, 4) AG in the antrum, and 5) AG in both antrum and corpus (pan-gastritis) (11,12). Thus, GastroPanel is optimized for use together with the Updated Sydney System (USS) for the classification of gastritis, which is based on these same five diagnostic categories (13). In addition, there are three other marker profiles specific to functional disturbances of the stomach, where morphology is normal (details in section 17).

GastroPanel has been validated in several large trials based on biopsy-confirmed gastroscopies (14,15), all included in a meta-analysis of the subject (16). These studies have been exploited to establish the validated reference (cut-off) values for each individual biomarker of the panel for the five histological endpoints. These studies also confirm the high accuracy of GastroPanel in detecting the most important endpoint, moderate-to-severe AG (14-16). Thus, normal values of PGI, PGII and their ratio (PGI/PGII) preclude AG of the corpus with NPV of over 95%. In turn, the values of PGI and PGII as well as their ratio below the established cut-off levels predict moderate-to-severe AG with area under ROC curve (AUC) values of above 0.950 in adequately-powered and USS-validated series (1, 2, 3, 16, 17).

In brief, the levels of PGI decrease in AG of the corpus (and in pan-gastritis), but remain within the normal range in all other conditions. Elevated PGII levels reflect mucosal inflammation, the highest values being detected in *Hp*-associated non-AG. The G-17b values are highest in AG of the corpus, because of the missing negative feedback by the acid output from an atrophic corpus, resulting in uninhibited secretion of G-17b by the normal antral mucosa. The same applies to the situation where acid output is inhibited by prolonged use of PPI medication. By definition, when antral mucosa is atrophic and the G cells are depleted, G-17 secretion remains very low, even after protein stimulation (G-17s)(17).

Hp IgG antibodies give significant added diagnostic value to the three biomarkers. IgG antibody level for *Hp* measures two potentially different conditions: 1) an ongoing *Hp* infection, or 2) a previous exposure to *Hp*. As the only abnormal marker, *Hp* implicates an *Hp*-associated superficial gastritis (non-AG), while associated with abnormalities in the other three markers, elevated *Hp* antibody levels confirm the diagnosis of *Hp*-associated AG (antrum or corpus) (1, 3, 18, 19).

The GastroPanel test can detect the following conditions:

1) *H. pylori* infection, which is an independent risk factor of both gastric cancer and peptic ulcer disease (gastric and duodenal ulcer).

2) *H. pylori*-induced atrophic gastritis (AG), which in most cases is asymptomatic, as well as the topographic site of AG either in the corpus and/or the antrum. Apart from *H. pylori*, AG in the corpus with all its clinical sequels can also develop through an autoimmune mechanism.

3) AG of the corpus, leading to low acid output or achlorhydric stomach. This increases the risk of gastric or esophageal cancer, as well as malabsorption of vitamin B12, calcium, magnesium and zinc. In addition, the absorption of some medicines, such as dipyridamol, some iron preparations and anti-fungal drugs (fluconazol, itraconazol), thyroxin and atazanovir is impaired due to an achlorhydric stomach. Calcium deficiency can cause osteoporosis, and vitamin B12 deficiency can contribute to the development of megaloblastic anemia, Alzheimer‘s disease, dementia, depression or peripheral neuropathies. Reduced acid output in the stomach can also increase the risk of serious infections in the gastrointestinal- and respiratory tract, including giardiasis, malaria, *Clostridium difficile, E. coli* EHEC, and pneumonia.

4) AG of the antrum, which increases the risk of peptic ulcer disease and gastric cancer. Co-existent AG of the corpus and antrum is the single most important risk condition for gastric cancer.

5) *H. pylori* infection also in subjects with AG, MALT-lymphoma or bleeding peptic ulcer, or when taking PPI medication or antibiotics. In these cases, 13C-urea breath tests (UBT) or stool *Hp* antigen tests frequently give false negative results and *H. pylori* infection (with all its consequences) remains undetected.

6) High acid output of the gastric mucosa, which predisposes to esophageal reflux disease with potential complications (ulcerative esophagitis, Barrett’s esophagus or lower esophageal cancer).

AG, high acid output and symptomatic *H. pylori* infection are indications for gastroscopy.

Globally, gastric cancer remains the third most common cause of cancer deaths and achlorhydric stomach is its most important risk factor. According to a recent meta-analysis, chronic use of PPI medication is also associated with an increased risk of gastric cancer (20). The common cause of both these conditions is the carcinogenic (Class I) acetaldehyde borne in achlorhydric stomach (21). Carcinogenicity of acetaldehyde is best documented by a human disease model, i.e., in exposed people who have mutations in the metabolizing enzyme, aldehyde dehydrogenase (ALDH), randomly distributed in some populations (22). This information is important, because disclosure of a specific carcinogenic substance enables taking the measures to reduce the exposure of the upper gastrointestinal tract to acetaldehyde on both population and individual levels (23). To accomplish this protection, it is recommended that all subjects with achlorhydric stomach, AG of the corpus and those on regular PPI medication should use Acetium-capsules to convert the carcinogenic acetaldehyde in the stomach into a harmless compound, thus reducing the risk of gastric and esophageal cancer (www.acetium.com).

For more details on the interpretation of GastroPanel results, please refer to Table 1 and www.gastropanel.com.

2. HELICOBACTER PYLORI AS PART OF GASTROPANEL®

GastroPanel is a quantitative enzyme-linked immunosorbent assay (ELISA) test panel that measures the blood plasma concentration of four biological markers of gastric mucosal structure and function: pepsinogen I (PGI), pepsinogen II (PGII), gastrin-17 (G-17) and *Helicobacter pylori* IgG antibodies. The indication for use for GastroPanel is to help in the diagnosis of symptomatic (dyspeptic) adult patients and for the screening of asymptomatic subjects to detect the risk groups of gastric cancer, i.e., those with 1) *H. pylori* infection 2) atrophic gastritis (AG). FOR *IN VITRO* DIAGNOSTIC USE.

3. INTENDED USE

GastroPanel *Helicobacter pylori* test is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of human IgG class antibodies to *Helicobacter pylori* in EDTA plasma. The test is intended for diagnosis of *Helicobacter pylori* infection in adult patients with upper abdominal symptoms (dyspepsia).The kit is used as part of GastroPanel. FOR *IN VITRO* DIAGNOSTIC USE.

4. HELICOBACTER PYLORI IgG BACKGROUND

Helicobacter pylori (*H. pylori*) infection is the most important cause of chronic gastritis leading to mucosal atrophy, i.e., atrophic gastritis (AG). A rarer cause of AG is an autoimmune mechanism (24, 25). This ELISA is intended for diagnosis of *H. pylori* infection in the plasma sample, based on IgG antibody detection.

H. pylori is a spiral-shaped, gram-negative bacterium that colonizes in the human stomach. The organism is found within the mucous layer overlying the gastric epithelium, and also within the mucosal glands, but it does not appear to invade the epithelial cells. However, the mucosa underneath and surrounding the areas of the *H. pylori* colonization is invariably inflamed; this condition is referred to as chronic superficial or non-atrophic gastritis which, if untreated, persists for life (1). Without adequate eradication of the bacteria, this chronic inflammatory process leads to AG. AG in turn increases the risk of peptic ulceration and gastric cancer, two important sequels of *H. pylori* infection (26-29). The presence of antibodies to *H. pylori* strains have been linked to the development of AG in the corpus (30). The epidemiological evidence indicates a link between *H. pylori* infection and gastric adenocarcinoma, as well as a mucosa-associated lymphatic tissue (MALT) lymphoma (18, 31, 32).

5. PRINCIPLE OF THE TEST

This GastroPanel *Helicobacter pylori* test is based on an enzyme immunoassay technique with partially purified *H. pylori* bacterial antigen adsorbed on a microplate and a detection antibody labeled with horseradish peroxidase (HRP).

The assay proceeds according to the following reactions:

1. Partially purified *H. pylori* bacterial antigen attached to the polystyrene surface of the wells binds *H. pylori* IgG antibodies present in the sample.
2. Wells are washed to remove residual sample.
3. HRP-conjugated monoclonal anti-human IgG binds to the *H. pylori* IgG antibodies.
4. The wells are washed and the TMB substrate is added. The substrate is oxidized by the HRP enzyme, resulting in the formation of a blue end product.

5. The enzyme reaction is terminated with the stop solution. *H. pylori* positive samples turn yellow with calculated values of >30 EIU.

6. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

CAUTION: Handle plasma samples as potential biohazardous material.

All samples should be regarded as potentially contaminated and treated as if they were infectious. Please refer to the U.S. Department of Health and Human Services (Bethesda, MD., USA) publication Biosafety in Microbiological and Biomedical Laboratories, 1999, 4th ed. (CDC/NIH) and No. (CDC) 88-8395 on reports of laboratory safety procedures on different diseases or any other local or national regulation.

This kit contains reagents manufactured from human blood components. The source materials provided in this kit have been tested for the presence of antibodies to hepatitis B and C, as well as for antibodies to HIV, and were found to be negative. However, as no test method can offer absolute assurance that these pathogens are absent, all recommended precautions for the handling of blood derivatives should be observed.
Always use protective gloves when handling patient samples. Use a safety pipetting device for all pipetting. Never pipette by mouth. Read all instructions prior to performing this assay.
Components containing ProClin may cause an allergic skin reaction (see Safety Data Sheet). Dispose of ProClin containing solutions according to local waste management legislation.

7. TRACEABILITY OF VALUES

There is no international reference material to *H. pylori* antigen. The calibrator and control values are assigned to Biohit internal master calibrators.

8. KIT CONTENTS, REAGENT PREPARATION AND STABILITY FOR MATERIALS PROVIDED

The reagents are sufficient for 96 wells and three separate runs. Reagents of different kit lots should not be mixed.

8.1. Microplate

Contents: 12 x 8 strips in frame coated with partially purified *H. pylori* bacterial antigen.

Preparation: Ready for use.

Stability: Stable until expiry date. Discard the strips after use.

8.2. Washing Buffer Concentrate (10x)

Contents: 120 ml of 10x phosphate buffered saline (PBS) concentrate containing Tween 20 and 0.1% ProClin 300 as preservative.

Preparation: Dilute 1 to 10 (e.g. 100 ml + 900 ml) with distilled water and mix well.

Stability: The concentrate is stable until expiry date. The diluted solution is stable for two weeks refrigerated (2-8°C).

8.3. Sample Diluent Buffer

Contents: 100 ml of phosphate buffer containing casein, Tween 20, 0.1% ProClin 300 as preservative and red dye.

Preparation: Ready for use.

Stability: Stable until expiry date.

8.4. Calibrators

Contents: Four vials containing 1.5 ml of human serum-based *H. pylori* IgG calibrator with 0.1% ProClin 300 as preservative. The EIU-value of the lot-specific calibrator is printed on the label of the vial.

Preparation: Ready for use.

Stability: Stable until expiry date.

8.5. Control

Contents: One vial containing 1.5 ml of human serum-based *H. pylori* IgG control with 0.1% ProClin 300 as preservative. The EIU-value of the control serum is indicated on the label.

Preparation: Ready for use.

Stability: Stable until expiry date.

8.6. Conjugate

Contents: 15 ml of HRP-conjugated monoclonal anti-human IgG in stabilizing buffer with 0.02% methylisothiazolone and 0.02% bromonitrodioxane, and 0.002% other active isothiazolones as preservatives.

Preparation: Ready for use.

Stability: Stable until expiry date.

8.7. Substrate Solution

Contents: 15 ml of tetramethylbenzidine (TMB) in aqueous solution.

Preparation: Ready for use.

Stability: Stable until expiry date. Avoid exposure to direct light.

8.8. Stop Solution

Contents: 15 ml of 0.1 mol/l sulfuric acid.

Preparation: Ready for use.

Stability: Stable until expiry date.

8.9. Incubation Covers

Three plastic sheets to cover the microplate during incubation.

8.10. Instructions for Use

Inserted into each kit.

9. SPECIMEN COLLECTION AND HANDLING

It is recommended that the blood sample is drawn after overnight fasting (approximately 10 hours), but at least after 4 hours of fasting, into an EDTA tube without additives. Blood tubes for plasma are mixed immediately by turning them upside down 5-6 times. Plasma is separated by centrifugation immediately or after 2 hours at the latest. (e.g., StatSpin® Express 2, centrifugation for 2 minutes at 4440 x g; please refer to centrifuge manufacturer instructions for plasma separation).

After separation of the plasma, add GastroPanel Stabilizer to the sample (50 µl/1 ml plasma; Biohit Oyj, GastroPanel Stabilizer, Cat. Nos. 606 050 and 606 051). The addition of the stabilizer into the plasma sample immediately after separation enables the storage of the sample for 7 days in a refrigerator at 2-8°C and 3 days at room temperature (20-25 °C).

9.1 Sample freezing

Freeze the sample immediately after separation and addition of GastroPanel Stabilizer. For temporary storage, the plasma samples can be stored frozen at -20 °C, but in long-term storage of over two weeks, the storage should be at -70 °C. Mix the samples thoroughly after thawing. Avoid repeated freezing and thawing of the samples. Grossly hemolyzed, lipemic, or turbid specimens should be discarded.

9.2 Gastrin-17 stimulation

When a postprandial, protein-stimulated blood sample is needed, a drink made from protein powder (Biohit Oyj, Cat. No. (601 037 or 601 038) should be taken after fasting for a minimum of 4-10 hours. Twenty minutes after the protein drink is consumed, blood is drawn into an EDTA tube.

10. MATERIALS REQUIRED BUT NOT PROVIDED

10.1 Manual method

Distilled or deionized water, micropipettes and disposable tips to accurately deliver 10 – 1000 µl, pipettes to accurately deliver 1-10 ml, 8-channel pipette delivering 100 µl, 1000 ml graduated cylinder, vortex mixer for sample dilutions, test tubes for specimen dilutions, microplate washer, paper towels or absorbent paper, timer, vertical measurement principle microplate reader 450 nm (33), e.g., plastic blood collection tube for EDTA plasma, container for ice-water bath, plate shaker.

10.2 Automates

Distilled or deionized water for washing buffer dilution. GastroPanel is automation friendly. No additional instruments, accessories or disposables are needed to carry out GastroPanel analysis with commercial ELISA automates with the vertical measurement principle microplate reader (33).

11. STORAGE AND STABILITY

Store the GastroPanel *Helicobacter pylori* kit refrigerated (2-8°C). When stored at these temperatures, the kit is stable until the expiration date printed on the box label and the label of each individual kit component. Do not freeze or expose the kit to high temperatures or store at above 8 °C when not in use. The substrate solution is sensitive to light. The microplate or individual strips should not be removed from the foil pouch until equilibrated to room temperature (20-25 °C). Unused strips must be returned to the foil pouch, sealed and stored at 2-8 °C.

Do not use reagents after the expiration date printed on the label. Do not use reagents from kits with different lot numbers or substitute reagents from kits of other manufacturers. Use only distilled or deionized water. The components of the kit are provided at precise concentrations. Further dilution or other alterations of the reagents may cause incorrect results.

Indication of Kit Deterioration

Liquid components should not be visibly cloudy or contain precipitated material. At 2-8 °C, the washing buffer concentrate may, however, partially crystallize, but the crystals will dissolve by mixing at room temperature (20-25 °C). The diluent buffer is slightly opaque. The calibrators and control may also seem slightly opaque. The substrate solution should be colorless or pale blue. Any other color indicates deterioration of the substrate solution.

12. TEST PROCEDURE

PRELIMINARY PREPARATIONS

Allow all reagents and the microplate to reach room temperature (20-25 °C). Dilute the washing buffer concentrate 1 to 10 (e.g., 100 ml + 900 ml) with distilled or deionized water. Frozen samples should be thawed fast in a room temperature water bath with occasional mixing. Once they are almost thawed, place them in a crushed ice bath. **Read the complete assay procedure before starting. It is recommended that calibrators and control are applied on the plate as duplicates. It is necessary to use calibrators and the control in each test run.**

Mix all the reagents well before use. Note! All Incubations may be performed at 20-30 °C (=ambient temperature), do not exceed the specified temperature.

12.1. Manual Method

Follow the sample dilution instructions below for simultaneous analysis of the whole GastroPanel.

STEP 1: SPECIMEN DILUTION

The sample diluent buffer, washing buffer, stop solution and substrate can be used interchangeably between the kits, if of the same lot. All other components of the kit are specific to each individual kit.

GastroPanel sample dilutions

Dilution	**Analyte**
1:5	G-17
1:20	PGI
1.20	PGII
1:400	*H. pylori*

Make three separate dilutions from the sample. An example of the dilutions is shown below:

1. To make G-17 dilution: dilute the mixed EDTA plasma sample 1:5 (e.g., 100 µl plasma + 400 µl diluent buffer). Mix the tube.
2. To make PGI and PGII dilution: dilute the above-made 1:5 dilution further 1:4 to make a 1:20 dilution (e.g., 180 µl 1:5 dilution + 540 µl diluent buffer). Mix the tube.
3. To make H. pylori dilution: dilute the above-made 1:20 dilution further 1:20 to make a 1:400 dilution (e.g., 20 µl 1:20 dilution + 380 µl diluent buffer). Mix the tube.

STEP 2: SAMPLE

Mix and pipette 100 µl of the blank solution (BS, for G-17, PGI and PGII) or sample diluent buffer (Blank, for *H. pylori*), calibrators, the control and diluted samples into the microplate wells (see Figure 1 for *H. pylori,* and Figures 2 and 3 for G-17 and PGI/PGII, respectively). You may cover the plate with the incubation cover to avoid splashes.

Incubate for 60 minutes at ambient temperature with shaking (750 rpm). Note: It is recommended that the samples are dispensed into the wells of one plate within 20 minutes to avoid assay drift within the plate.

	1	2	3	4
A	Blank	Blank	Sample	Sample
B	CAL 1	CAL 1	etc.	etc.
C	CAL 2	CAL 2		
D	CAL 3	CAL 3		
E	CAL 4	CAL 4		
F	Control	Control		
G	Sample	Sample		
H	Sample	Sample		

Figure 1. Pipetting order of H. pylori

	1	2	3	4
A	BS	BS	etc.	etc.
B	CAL1	CAL1		
C	CAL2	CAL2		
D	CAL3	CAL3		
E	CAL4	CAL4		
F	Control	Control		
G	Sample	Sample		
H	Sample	Sample		

Figure 2. Pipetting order of G-17

	1	2	3	4
A	BS	BS	etc.	etc.
B	CAL1	CAL1		
C	CAL2	CAL2		
D	CAL3	CAL3		
E	Control	Control		
F	Sample	Sample		
G	Sample	Sample		
H	Sample	Sample		

Figure 3. Pipetting order of PGI and PGII

STEP 3: WASHING

Wash the microplate strips with 3 x 350 µl of the diluted (1 to 10) washing buffer and gently tap the inverted plate a few times on a clean paper towel.

STEP 4: CONJUGATE

Note! Each individual kit has its specific conjugate (not interchangeable). Pipette 100 µl of the conjugate solution into the emptied microplate wells with an 8-channel pipette. You may cover the plate with the incubation cover. Incubate for 60 minutes at ambient temperature with shaking (750 rpm).

STEP 5: WASHING

Wash the microplate strips with 3 x 350 µl of the diluted (1 to 10) washing buffer and gently tap the inverted plate a few times on a clean paper towel.

STEP 6: SUBSTRATE

Pipette 100 µl of the substrate solution into the microplate wells with an 8-channel pipette. Start the incubation time after pipetting the substrate solution into the first microplate strip and continue the incubation for 30 minutes at ambient temperature. Avoid direct exposure to light during incubation.

STEP 7: REACTION STOP

Pipette 100 µl of the stop solution with an 8-channel pipette into the microplate wells.

STEP 8: MEASURING OF RESULTS BY VERTICAL MEASUREMENT PRINCIPLE

Measure the absorbance of microplate wells at 450 nm within 30 minutes (33).

12.2. Automated method

GastroPanel has been designed with automation in mind. As soon as test specific protocols have been created and validated for use, running the GastroPanel with a walk-away open ELISA automate saves on resources, and is easy and user friendly, e.g., by avoiding pipetting-induced disorders such as RSI.

The only manual step needed is to prepare a 1:10 dilution of the washing buffer concentrate before the next run. The whole assay process, from sample dilution up to the final result calculation and reporting, is performed automatically from start to finish.

13. RESULTS

13.1. Quality Control Values

Good Laboratory Practice requires the use of appropriate controls to establish that all the reagents and protocols are performing as designated. The GastroPanel *Helicobacter pylori* is provided with the control. Quality control charts within the lot should be maintained to follow the performance of the control. Alternatively, appropriate statistical methods may be used for analyzing internal laboratory control values, which should fall within the appropriate confidence intervals employed in each laboratory. The expected control results must be obtained so that the results can be accepted.

13.2. Calculation of the Results

The absorbance readings are converted to *H. pylori* IgG immunounits (EIU) by interpolating unknowns from the best-fit curve of the calibrators. Since the calibrators are ready to use, the concentrations of the patient samples are not multiplied by the dilution factor.

Subtract the mean OD of the blank from all OD values of the wells. Plot the mean OD of the calibrators vs their respective EIU value. Apply logarithmic fit to interpolate the unknown concentrations. A typical calibration curve is shown in Figure 4.

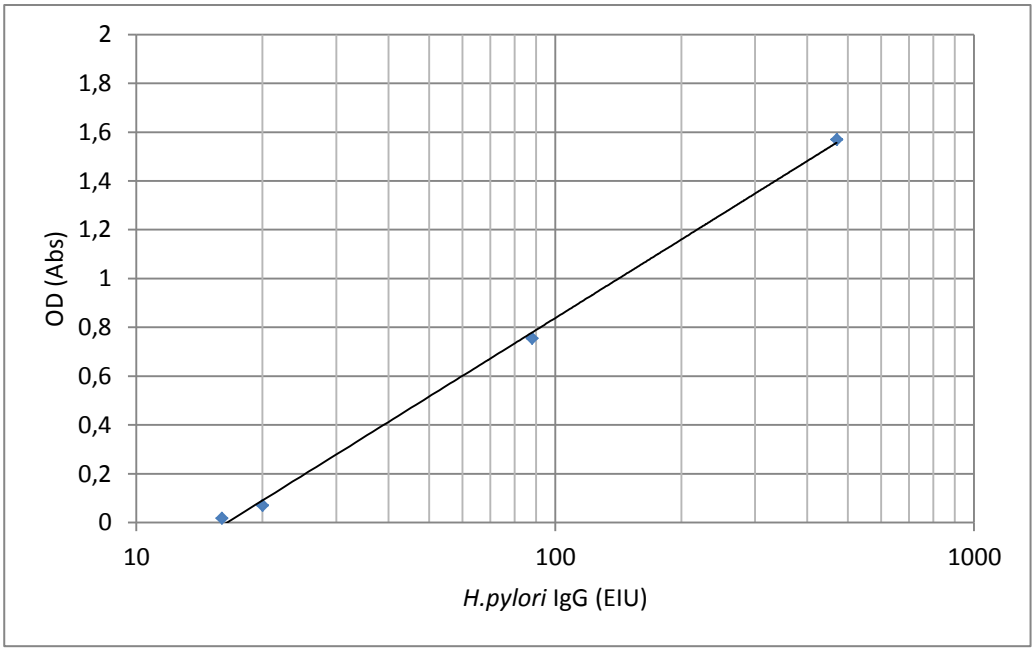


Figure 4. Example of a Typical H. pylori Calibrator Curve.

As the interpretation should be based on all the GastroPanel markers measured from the same patient sample, assay data must be gathered and analyzed together, with optional anamnestic information such as use of PPI medication and information about *H. pylori* eradication.

Please refer to section 17 regarding interpretation. Should you be willing to automate GastroPanel interpretation, please contact Biohit for more information about the software applications and services. More information is also available on the GastroPanel product site (www.gastropanel.com).

13.3. Interpretation of the Results

Negative	< 30 EIU
Positive	≥ 30 EIU

A value of less than 30 EIU indicates a negative result, i.e., implicating that no active *H. pylori* infection exists. A value of 30 EIU or over indicates that *H. pylori* antibodies are detected, and the result is thus positive. Expected control results must be obtained in order to accept the test results. The cut-off values have been determined using the GastroPanel *Helicobacter pylori* kit. From patients whose samples yield values near the cut-off, a second sample should be collected, if possible, within a reasonable time period. Each laboratory may establish its own range of expected values for the clinical situation where *H. pylori* IgG antibody values are used for diagnosis. In addition, the *H. pylori* IgG results determined for a given specimen or with assays from different manufacturers may vary due to differences in assay methods and reagent specificity. Results obtained by other assay methods from other manufacturers should not be used interchangeably.

13.4. Biological reference interval

The cut-off value is 30 EIU, with a reference interval of < 30 EIU. The interval is based on 7000 Finnish subjects (Biohit internal report, unpublished data).

Most persons exposed to *H. pylori* develop IgG antibodies to the organism (25, 28, 29). Age-specific rates for the presence of *H. pylori* antibodies are similar for males and females. The prevalence of *H. pylori* infection is 30-40% in the U.S., Canada, and in Western Europe, about 20% in Australia, and 70-90% in Eastern Europe, Africa, South America and Asia. Many patients with elevated *H. pylori* antibody levels are asymptomatic, even though they are colonized with *H. pylori* (15, 18). Therefore, antibody levels do not necessarily correlate with the severity of clinical symptoms.

14. LIMITATIONS OF THE PROCEDURE

As with any diagnostic procedure, the GastroPanel *Helicobacter pylori* results must be interpreted together with the patient's clinical presentation and any other information available to the physician. Given the long decay (months) of serum IgG antibodies, the test can give (false) positive results (*H. pylori* >30 EIU) shortly after the successful eradication of *H. pylori* infection.

15. ANALYTICAL PERFORMANCE CHARACTERISTICS

All performance tests were carried out at room temperature (20-25 °C). All samples were analyzed with duplicate microplate wells.

Measuring range:

The measuring range for GastroPanel *Helicobacter pylori* is from 15 EIU to 670 EIU.

In this range, repeatability has been demonstrated to be ≤ 10 CV%, within-assay precision ≤ 20 CV% and total error at LoQ level ≤ +/- 20%.

Precision:

The precision studies were performed according to the CLSI EP5-A2 guidelines. A panel consisting of seven EDTA plasma samples over various levels of low, mid and high concentrations of anti-*H. pylori* antibodies were run in duplicates on 20 operation days (two runs per day, two repeats per sample per run). Three production lots, seven operators, and two instruments were employed. Statistical analysis was performed in agreement with the CLSI EP5-A2 guidelines to determine the estimates of repeatability (within-run) precision and within-laboratory precision.

In the repeatability precision for EDTA plasma samples, the range was from 16.6 EIU to 669 EIU, the standard deviations from 0.59 EIU to 64.95 EIU, and the %CV from 3.6% to 9.8%. In the within-laboratory precision for EDTA plasma, the standard deviation range was from 0.89 EIU to 107.37 EIU, and the %CV from 5.7% to 16.5%.

REPEATABILITY					
Sample	**Mean (EIU)**	**%CV**	**Total SD**	**95% CI**	**n**
1	16.6	3.60%	0.59	0.485 to 0.756	80
2	22.9	3.70%	0.85	0.695 to 1.084	80
3	38.2	5.10%	1.96	1.605 to 2.502	78
4	72.6	6.10%	4.42	3.626 to 5.651	78
5	133.5	7.80%	10.36	8.505 to 13.255	78
6	261	9.80%	25.6	21.018 to 32.756	80
7	669	9.70%	64.95	53.322 to 83.099	78
WITHIN-LABORATORY					
Sample	**Mean (EIU)**	**%CV**	**Total SD**	**95% CI**	**n**
1	16.6	5.40%	0.89	0.743 to 1.102	80
2	22.9	6.20%	1.42	1.201 to 1.746	80
3	38.2	7.90%	3.01	2.558 to 3.667	78
4	72.6	13.30%	9.63	7.999 to 12.085	78
5	133.5	16.50%	22.09	18.449 to 27.522	78
6	261	16.50%	43.06	35.673 to 54.341	80
7	669	16.00%	107.37	90.141 to 132.790	78

Detection limit and quantitation limit:

The limit of blank (LoB) and the limit of detection (LoD) for GastroPanel *Helicobacter pylori* was determined consistent with the CLSI Guideline EP17-S with proportions of false positive (α) less than 5% and false negatives (β) less than 5%, based on 120 determinations with 60 samples close to blank and 60 low level samples. Four EDTA plasma samples and three kit lots were used to establish the LoD and kit diluent buffer for establishing the LoB.

The LoB was found to be 13.1 EIU and the LoD 14.7 EIU pmol/l.

The limit of quantitation was determined consistent with the NCCLS Guideline EP17-S based on 60 determinations of four EDTA plasma samples with three kit lots. Due to the lack of a reference method, the bias estimation was not included in total error calculations.

The LoQ was found to be 15.0 EIU with a total error of -10.7% and with a CV% between measurements of 5.2%.

Interference:

GastroPanel *Helicobacter pylori* was evaluated for interference according to CLSI Guideline EP07-A2. The bias caused by hemoglobin, unconjugated bilirubin, conjugated bilirubin and triglycerides at concentrations of 2 g/l, 5 mg/dl, 15 mg/dl and 500 mg/dl, respectively, was found to be less than 10% at *H. pylori* IgG plasma levels of 21 EIU and 70 EIU. This was considered a non-significant interference. Grossly hemolyzed, lipemic, or turbid specimens should be discarded.

16. DIAGNOSTIC PERFORMANCE

The cohort of the validation trial consisted of 101 gastroscopy referral patients of Caucasian origin, including 71 women and 30 men. The mean age of the study subjects was 50.1 years, SD=16.7 years, and range 18-83 years.

ROC analysis for biopsy-confirmed *H. pylori* by the GastroPanel *Helicobacter pylori* test gave AUC=0.978 (95% CI 0.956-1.000). The best sensitivity/specificity (SE/SP) balance is 90.8% SE and 88.6% SP, corresponding to the cut-off level of 30 EIU.

17. INTERPRETATION OF THE GASTROPANEL® RESULTS

GastroPanel is optimized for use in context with the Updated Sydney System (USS) for the classification of gastritis. Both the USS and the GastroSoft® software use five diagnostic categories to classify the biopsy and the GastroPanel results, respectively. These include: 1) normal mucosa, 2) superficial (*Hp*) gastritis, 3) AG in the antrum, 4) AG in the corpus, and 5) AG in both antrum and corpus (pan-gastritis) (13, 34, 35). In addition to these five categories related to stomach morphology, three other marker profiles are possible in GastroPanel, being specific for defined functional disturbances with normal stomach morphology. All eight diagnostic categories are depicted in Table 1, and explained in the following.

17.1 Healthy stomach

With all four biomarkers within the normal reference range, gastric mucosa functions normally. Given that the function of stomach mucosa is critically dependent on the specific cells responsible for acid output (parietal cells), pepsinogens (chief cells) and G-17 (G cells), normal function necessitates the presence of these cells in normal quantities (1, 3, 9, 11, 19). Thus, stomach function and mucosal structure go hand-in-hand, and by definition, a normal GastroPanel result is a surrogate marker of a healthy stomach.

17.2 High acid output

Gastric acid (HCl) is produced by the highly specialized parietal cells in the corpus. Acid output is controlled, among other things, by the secretion of G-17 in the antrum, as a result of a positive feedback loop stimulating acid output after a meal. Acid output results in progressively lower pH in the corpus, and the threshold of pH 2.5 triggers a negative feedback to antral G cells, signaling them to down-regulate the output of G-17. As a result, G-17 output decreases in parallel with the acid content of the corpus (1, 3, 14, 17). When, due to any reason, acid output in the corpus remains abnormally high (other stimulatory mechanisms), the end result is abnormally low G-17b output from the antral G cells. This condition is best diagnosed by the test medication with PPI, when the G-17b should normalize within approximately two weeks of therapy. Under these circumstances, postprandial (stimulated) G-17s will be within normal limits, because the G cells are intact and capable of G-17 secretion when properly stimulated (protein powder, Biohit Cat. No. 601038).

17.3. Low acid output due to Proton Pump Inhibitor (PPI) medication

The regulation above also works the other way round. When acid output in the corpus is reduced (for any reason), the positive feedback loop triggers antral G cells to increase their G-17b secretion, resulting in elevated serum levels of G-17b (3, 17). The two conditions with low acid output are 1) AG in the corpus, and 2) long-term use of PPI medication. The former is excluded by the normal (or even elevated) values of PGI, PGII, and normal PGI/PGII ratio, while the latter is best diagnosed by discontinuing the PPI medication. In that case, the antral G-17b should be normalized within two weeks (17.8).

17.4. Superficial (non-atrophic), *Helicobacter pylori*-associated gastritis

Like all bacteria, *Helicobacter pylori* will also induce acute inflammation in the gastric mucosa, with a usual onset in the antrum (1, 3, 7, 13, 18, 36). Three different marker profiles can be encountered in association with *Hp* infection.

17.4a In an active *Hp* infection, *Hp* antibody levels are raised, which can be the only abnormal finding in GastroPanel, with all other markers falling within a normal range. Not infrequently, however, an active ongoing *Hp* infection causes a severe inflammatory reaction which, due to increased cell permeability, can lead to increased leakage of PGI, PGII, and even G-17 from the cells and result in elevated serum levels of any or all of these three biomarkers (3, 7, 36).

17.4b Successful *Hp* eradication by active treatment should result in normalized values of all three markers, however, with a delay of some weeks to months. *Hp* antibody levels can remain elevated for a longer period of time, which is unpredictable and limits the usefulness of GastroPanel as an accurate diagnostic test for the control of *Hp* eradication (36).

17.4c In cases where *Hp* eradication attempt fails, *Hp* antibody levels remain elevated (usually slightly), and the PGI and PGI/PGII ratio usually falls within a normal range, whereas PGII and/or G-17b may be slightly elevated due to ongoing inflammatory reaction (see 17.4a). The result can be confirmed after 5-6 months, followed by a new treatment attempt if indicated (3, 36).

17.5 Atrophic gastritis of the corpus

By definition, the loss of specific cells (chief cells) in the oxyntic glands of the corpus mucosa as a result of mucosal atrophy will lead to a progressively reduced output of PGI and (to a lesser extent) PGII, which is also produced by the same cells in the antral mucosa. This disproportionate reduction of these two markers will result in reduced PGI/PGII ratio, which is another excellent signature of AG in the corpus (1, 3, 5-9, 14, 16). This reduction of PGI and PGI/PGII ratio is progressive and closely correlated with the severity of corpus atrophy, with total atrophy and acid-free stomach as the end point. In the case of intact (normal) antral mucosa, this leads to markedly increased output and serum levels of G-17b (17, 19). There is no need to test G-17s in such a situation. In chronic cases with a protracted course, *Hp* may disappear, resulting in gradual normalization of *Hp* antibody levels.

17.6 Atrophic gastritis of the antrum

When the mucosal atrophy only affects the antrum, all corpus-specific markers will be within the normal range. By definition, AG in the antrum is caused by *Hp* infection, and *Hp* antibodies are invariably elevated in GastroPanel testing. As a result of antrum atrophy, G cells are reduced in number and finally disappear, leading to progressively reduced plasma levels of G-17b. In severe antrum atrophy, there is no response to protein

stimulation of G-17s secretion because of the lack of (target) G cells in the mucosa (14, 15, 17).

17.7 Atrophic gastritis of the antrum and corpus

The most severe form of AG is known as atrophic pan-gastritis, affecting both the antrum and corpus. As an end result, the specified cells (chief cells) in the corpus and antrum (G cells) disappear, leading to a biomarker expression pattern where both pepsinogens (PGI, PGII) and G-17 are substantially reduced (1, 3, 5-9, 14, 16, 17, 19). This applies to both G-17b and G-17s, which remain low even after stimulation because of the missing G cells. Like in AG of the corpus (17.5), *Hp* antibody levels can be normal or elevated. This is because in chronic AG, *Hp* can disappear in the atrophic mucosa, and in the absence of antigen stimulus, a normal decay of IgG antibodies will reduce the *Hp* antibody levels to be below the 30 EIU cut-off level.

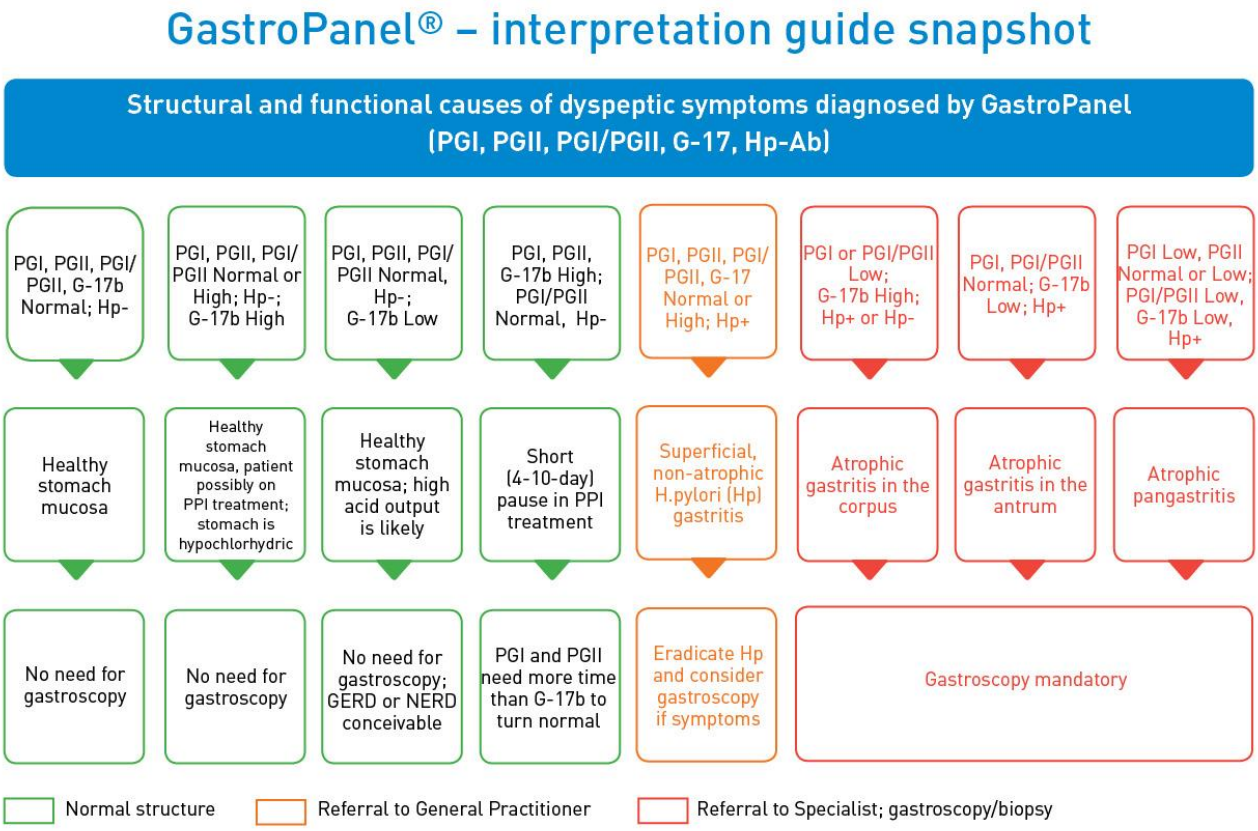
17.8 PPI medication

If the patient uses any PPI gastric acid suppression medication, please contact the person taking the samples. Moreover, enter the information in the patient's case history, as it will be included in the GastroSoft printout. Proton pump inhibitors (PPI) reduce gastric acid production in the stomach. This increases the production of gastrin-17, increasing pepsinogen levels. Once the PPI treatment is completed, it takes approximately 4–10 days for hydrochloric acid production and gastrin-17 levels to return to normal. However, pepsinogen levels will remain high for a relatively long period. The cessation of long-term PPI acid suppression is typically followed by rebound acid hypersecretion (within 7–10 days), which means heartburn symptoms will return in force and gastrin-17 levels will be very low. (1, 3, 11, 17)

Table 1. The eight diagnostic categories of GastroPanel

	GastroPanel Biomarkers						Interpretation
	Pepsinogen I (30-160 µg/l)[@]	Pepsinogen II (3-15 µg/l)	PGI/PGII ratio (3-20)	Gastrin-17b (1-7 pmol/l)	Gastrin-17s (3-30 pmol/l)	*H. pylori* IgG Antibody level (<30 EIU)	
1	N	N	N	N	N	N	Healthy mucosa (no atrophy, no *H. pylori* infection)
2	N	N	N	L*	N	N	Healthy mucosa. High acid output in the corpus
3	N or H^	N or H^	N	H**	N	N	Healthy mucosa. Low acid output due to, e.g., PPI medication
4a	N or H^	N or H^	N	N or H^	ND	H	Active *H. pylori* infection, not treated
4b	N	N	N	N	ND	N or H[†]	*H. pylori* infection successfully eradicated
4c	N	H	N	H	ND	H	*H. pylori* eradication failed
5	L	L	L	H	ND	N^^ or H	Atrophic gastritis in the corpus
6	N	N	N	L	L	H	Atrophic gastritis in the antrum
7	L	L	L	L	L	N^^ or H	Atrophic gastritis in the antrum and corpus (pan-gastritis)
8	H	H	N	H	ND	N	Short (4-10day) break in PPI treatment

N=normal; L=low; H=high; *Test PPI medication for two weeks, G17b should normalize; **Stop PPI medication, G-17b should normalize in two weeks; ND, no need for testing; ^PGI, PGII and G-17 can be elevated due to mucosal inflammation; ^^*H. pylori* antibodies can disappear in mucosal atrophy with prolonged course; [@]Pepsinogen I cut-off value 30 µg/l is consonant with moderate/severe atrophic gastritis; [†]*H.pylori* antibody levels can remain elevated for months after successful eradication of *H.pylori*.



H. pylori infection- or autoimmune atrophic gastritis (AG), with associated risk of gastric cancer and other sequels, or the level of acid output in the stomach, cannot be diagnosed by the conventional tests used for diagnosis of dyspepsia and *H. pylori* infection, e.g., the 13C-urea breath test (UBT), or the stool antigen or antibody test. In subjects with AG, MALT-lymphoma or bleeding peptic ulcer, and in those on PPI medication or antibiotics, UBT or stool antigen tests frequently give false negative results, and *H. pylori* infection (with all its risks) remains undetected (37-41) (www.biohithealthcare.com/additional-information).

GastroPanel is capable of diagnosing atrophic gastritis affecting either the corpus or antrum or both. When compared with gastroscopy, accurate diagnosis of atrophic gastritis is not always possible in a few small biopsy specimens representing only a minimal sample of the adult gastric mucosal area. In addition, mucosal atrophy (mild atrophy in particular) is a subjective diagnosis, with substantial inter-observer variation among pathologists. Similarly, the accuracy of gastroscopy is dependent on the experience and competence of the gastroscopist. GastroPanel is not affected by these shortcomings, because it is an automated ELISA-based laboratory assay. In fact, endoscopic biopsy histology is not a reliable gold standard (42), although it is currently used as such. Compared with serum biomarkers, its limitations in diagnostic accuracy should be kept in mind (2, 43).

When performed by skillful gastroenterologists and pathologists, the agreement between GastroPanel and gastric biopsy histology is very good, exceeding 0.8 (the limit of almost perfect) by weighted kappa test (14). Importantly, the diagnosis of gastric atrophy is highly subjective without the use of gastric biopsies, i.e., on the basis of gastroscopy alone (44). When GastroPanel indicates that gastric mucosa is healthy (no *H. pylori* infection and/or

no atrophic gastritis), the clinical symptoms are often caused by functional dyspepsia or other functional disturbance without an organic disease in the gastric mucosa.

18. REFERENCES

1. Agréus L, Kuipers EJ, Kupcinskas L, Malfertheiner P, Di Mario F, Leja M, Mahachai V, Yaron N, van Oijen M, Perez Perez G, Rugge M, Ronkainen J, Salaspuro M, Sipponen P, Sugano K, Sung J. Rationale in diagnosis and screening of atrophic gastritis with stomach-specific plasma biomarkers. Scand J Gastroenterol 2012;47:136-147.

2. Storskrubb T, Aro P, Ronkainen J, Sipponen P, Nyhlin H, Talley NJ. Serum biomarkers provide an accurate method for diagnosis of atrophic gastritis in a general population: the Kalixanda study. Scand J Gastroenterol 2008;43:1448-1455.

3. Wikström .: Assessment of stomach health by “chemical gastroscopy”. Eur Gastroenterol Rev 2012;1-6.

4. Lomba-Viana R, Dinis-Ribeiro M, Fonseca F, Vieira AS, Bento MJ, Lomba-Viana H. Serum pepsinogen test for early detection of gastric cancer in a European country. Eur J Gastroenterol Hepatol 2012;24:37-41.

5. Miki K. Gastric cancer screening using the serum pepsinogen test method. Gastric Cancer 2006;9:245-253.

6. Bornschein J, Selgrad M, Wex T, Kuester D and Malfertheiner P. Serological assessment of gastric mucosal atrophy in gastric cancer. BMC Gastroenterol 201212:10. doi: 10.1186/1471-230X-12-10.

7. Germaná B, Di Mario F, Cavallaro LG, Moussa AM, Lecis P, Liatoupolou S, Comparato G, Carloni C, Bertiato G, Battiestel M, Papa N, Aragona G, Cavestro GM, Iori V, Merli R, Bertolini S, Caruana P, Franzé A. Clinical usefulness of serum pepsinogens I and II, gastrin-17 and anti-Helicobacter pylori antibodies in the management of dyspeptic patients in primary care. Dig Liver Dis 2005;37:501-508.

8. Miki K, Ichinose M, Shimizu A, Huang SC, Oka H, Furihata C, Matsushima T, Takahashi K. Serum pepsinogens as a screening test of extensive chronic gastritis. Gastroenterol Jpn 1987;22:133-141.

9. Saoff IM, Varis K, Ihamaki T, Siurala M, Rotter JI. Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. Gastroenterol 1982:83:204-209.

10. Korstanje A, den Hartog G, Biemond I, Lamers CB. The serological gastric biopsy: a non-endoscopical diagnostic approach in management of the dyspeptic patient: significance for primary care based on a survey of the literature. Scand J Gastroenterol 2002 236 (Suppl): 22–26.

11. Oksanen A, Sipponen P, Miettinen A, Sarna S, Rautelin H. Evaluation of bood tests to normal gastric mucosa. Scand J Gastroenterol 2000;35:791–795.

12. Varis K, Sipponen P, Laxen F, Samloff IM, Huttunen JK, Taylor PR, Helsinki Gastritis Study Group. Implications of serum pepsinogen I in early endoscopic diagnosis of gastric cancer and dysplasia. Scand J Gastroenterol 2000:35:950–956.

13. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161-1181.

14. Väänänen H, Vauhkonen M, Helske T, et al. Non-endoscopic diagnosis of atrophic gastritis with a blood test. Correlation between gastric histology and serum levels of gastrin-17 and pepsinogen I: a multicenter study. Eur J Gastroenterol Hepatol 2003:15:885–891.

15. Telaranta-Keerie A, Kara R, Paloheimo L, Härkönen M, Sipponen P: Prevalence of undiagnosed advanced atrophic corpus gastritis in Finland: an observational study among 4,256 volunteers without specific complaints. Scand J Gastroenterol 2010;45:1036-1041.

16. Dinis-Ribeiro M, Yamaki G, Miki K, Costa-Pereira A, Matsukawa M, Kurihara M: Meta-analysis on the validity of pepsinogen test for gastric carcinoma, dysplasia or chronic atrophic gastritis screening. J Med Screen 2004;11:141–147.

17. Sipponen P, Ranta P, Helske T, Kääriäinen I, Mäki T, Linnala A. Serum levels of amidated gastrin-17 and pepsinogen I in atrophic gastritis: an observational case-control study. Scand J Gastroenterol 2002;37:785–791.

18. Benberin V, Bektayeva R, Karabayeva R, Lebedev A, Akemeyeva K, Paloheimo L, Syrjänen K. Prevalence of H.pylori infection and atrophic gastritis among symptomatic and dyspeptic adults in Kazakhstan. A hospital-based screening with a panel of serum biomarkers. Anticancer Res 2013;33:4595-4602.

19. Syrjänen KJ, Sipponen P, Härkönen M, Peetsalu A, Korpela S. Accuracy of GastroPanel testing in detection of atrophic gastritis. Eur J Gastroenterol Hepatol 2015;27:102-104.

20. Ahn JS, Eom C-S, Jeon CY, Park MS. Acid suppressive drugs and gastric cancer: A meta-analysis of observational studies. World J Gastroenterol 2013;19:2560-8.

21. In IARC monographs on the evaluation of carcinogenic risk to humans. Personal habits and indoor combustions volume 100E, 4.3.2 The Role of acetaldehyde in alcohol-induced carcinogenesis, 2012. Pp. 471. Available: http://monogpahs.iarc.fr./ENG/Monographs/vol/100E.

22. Maejima R, Katsunori I, Kaihovaara P, Hatta W, Koike T, Imatani A, Shimosegawa T, Salaspuro M. Effects of ALDH2 genotype, PPI treatment and L-cysteine on carcinogenic acetaldehyde in gastric juice and saliva after intragastric alcohol administration. PLoS One. 2015; 10(4): e0120397.

23. Salaspuro M. Acetaldehyde and gastric cancer. J Dig Dis 2011; 12:51-59. doi: 10.1111/j.1751-2980.2011.00480.xPMID:21401890.

24. Varis K, Sipponen P. Gastritis. In: Principles and Practice of Gastroenterology and Hepatology. Gitnick G (ed.). Appleton & Lange, Connecticut, 1994; 85-197.

25. Sipponen P. *Helicobacter pylori* gastritis-epidemiology. J Gastroenterol 1997; 32:273-277.

26. Sipponen P, Marshall BJ. Gastritis and gastric cancer. Western countries. Gastroenterol Clin North Am 2000; 29:579-592.

27. Wadström T. An update on *Helicobacter pylori* Current Opinion in Gastroenterology 1995; 11:69-75.

28. Northfield TC, Mendall M, Goggin PC. *Helicobacter pylori* Infection, Pathophysiology, Epidemiology and Management. Kluwer Academic Press; Dordrecht: 1994.

29. Sipponen P. Update on the Pathologic Approach to the Diagnosis of Gastritis, Gastric Atrophy, and *Helicobacter pylori* and its sequalae. J Clin Gastroenterol 2001; 32:96-202.

30. Sande N, Nikulin M, Nilson I, Wadström T, Laxen F, Härkönen M, Suovaniemi O, Sipponen P. Increased Risk of Developing Atrophic Gastritis in Patients Infected with CagA+ *Helicobacter pylori*. Scand. J Gastroenterol 2001; 36:928-933.

31. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 1991; 325:1127-1131.

32. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Friedman GD. *Helicobacter pylori* infection and gastric lymphoma. N Engl J Med 1994; 330:1267-1271.

33. www.biohithealthcare.com/About US/History: Aggressive innovation and patenting strategy. www.biohithealthcare.com/Scientific/Litterature/Suovaniemi O: Automated instrumentation for clinical and research laboratories

34. Misiewicz JJ. The Sydney System: a new classification of gastritis. Introduction. J Gastroenterol Hepatol. 1991; 6:207–208.

35. Sipponen P, Price AB. The Sydney System for classification of gastritis 20 years ago. J Gastroenterol Hepatol. 2011;26: Suppl 1:31-34.

36. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. European Helicobacter Study Group. Management of Helicobacter pylori infection - the Maastricht IV/ Florence Consensus Report. Gut 2012;61:646-664.

37. Graham DY, Opekun AR, Hammoud F, Yamaoka Y, Reddy R, Osato MS, El-Zimaity HM. Studies regarding the mechanism of false negative urea breath tests with proton pump inhibitors. Am J Gastroenterol. 2003;98(5):1005-1009.

38. Savarinoa V, Vignerib S, Cellea G. The 13C urea breath test in the diagnosis of Helicobacter pylori infection. Gut 1999;45:118-122 doi:10.1136/gut.45.2008.i18.

39. Kokkola A, Rautelin H, Puolakkainen P, Sipponen P, Färkkilä M, Haapiainen R, Kosunen TU. Diagnosis of Helicobacter pylori infection in patients with atrophic gastritis: comparison of histology, 13C-urea breath test, and serology. Scand J Gastroenterol. 2000;35(2):138-141.

40. Gisbert JP, Esteban C, Jimenez I, Moreno-Otero R. 13C-urea breath test during hospitalization for the diagnosis of Helicobacter pylori infection in peptic ulcer bleeding. Helicobacter 2007; 12(3):231-237.

41. Megraud F, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. Clin Microbiol Rev. 2007;20(2):280–322.

42. Iijima K, Abe Y, Kikuchi R, Koike T, 1. Ohara S, Sipponen P, Shimosegawa T. Serum biomarker tests are useful in delineating between patients with gastric atrophy and a normal, healthy stomach. World J Gastroenterol 2009;15 (7):853-859.

43. Ren JS, Kamangar F, Qiao YL, Taylor P, Liang H, Dawsey S, Liu B, Fan JH, Abnet C. Serum pepsinogens and risk of gastric and oesophageal cancers in the General Population Nutrition Intervention Trial cohort. Gut 2009;58:636–42. doi:10.1136/gut.2008.168641.

44. Yanaoka K, Oka M, Yoshimura N, Mukoubayashi C, Enomoto S, Iguchi M, Magari H, Utsunomiya H, Tamai H, Arii K, Yamamichi N, Fujishiro M, Takeshita T, Mohara O, Ichinose M. Risk of gastric cancer in asymptomatic, middle-aged Japanese subjects based on serum pepsinogen and Helicobacter pylori levels. Int J Cancer 2008;123: 917 – 926.

19. DATE OF ISSUE

GastroPanel® *Helicobacter pylori* kit insert.
Version 4.0, September 2016.

20. WARRANTY

The Manufacturer shall remedy all defects discovered in any Product (the “Defective Product”) that result from unsuitable materials or negligent workmanship and which prevent the mechanical functioning or intended use of the Products including, but not limited to, the functions specified in the Manufacturer’s specifications for the Products. ANY WARRANTY WILL, HOWEVER, BE DEEMED VOID IF FAULT IS FOUND TO HAVE BEEN CAUSED BY MALTREATMENT, MISUSE, ACCIDENTIAL DAMAGE, INCORRECT STORAGE OR USE OF THE PRODUCTS FOR OPERATIONS OUTSIDE THEIR SPECIFIED LIMITATIONS OR OUTSIDE THEIR SPECIFICATIONS, CONTRARY TO THE INSTRUCTIONS GIVEN IN THE INSTRUCTION MANUAL.
The period of this warranty for the Distributor is defined in the instruction manual of the Products and will commence from the date the relevant Product is shipped by the Manufacturer. In case of interpretation disputes the English text applies.

All Biohit diagnostic kits have been manufactured according to ISO 9001/ISO 13485 quality management protocols and have passed all relevant Quality Assurance procedures related to these products.

21. ORDERING INFORMATION

GastroPanel®

Cat. No. 606 400.

Headquarters
BIOHIT OYJ
Laippatie 1
00880 Helsinki, Finland
Tel: +358-9-773 861
Fax: +358-9-773 2867
E-mail: info@biohit.fi
www.biohithealthcare.com

NOTES

22. BRIEF OUTLINE OF THE PROCEDURE

Allow all the reagents to reach ambient temperature. Remember to mix all the reagents and samples well just before pipetting

*

After mixing, pipette 100 µL of the diluent buffer (blank), the calibrators 1-4, the control, and diluted (1 to 400) patient samples into the wells

*

Incubate for **60 min at ambient temperature with shaking (750 rpm)**

*

Wash the strips with 3 x 350 µL of the diluted washing buffer

*

Pipette 100 µL of the mixed conjugate solution into the wells

*

Incubate for **60 min at ambient temperature with shaking (750 rpm)**

*

Wash the wells three times with 350 µl of the diluted washing buffer

*

Pipette 100 µL of the mixed substrate solution into the wells

*

Incubate for **30 min** at ambient temperature

*

Pipette 100 µL of the mixed stop solution into the wells

*

Read **at 450 nm** within 30 minutes