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GastroPanel Four-in-One™

ELISA kit for the measurement of human pepsinogen I, pepsinogen II, gastrin-17, and human IgG antibodies to *Helicobacter pylori* in EDTA plasma as part of GastroPanel

INSTRUCTIONS FOR USE

REF 606080

IVD

CE

For *in vitro* diagnostic use

Store at 2-8 °C upon receipt

GastroPanel® Product Family

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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
	Storage limitation. Store at 2-8 °C
	4x24 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
BLANK	Blank solution

INSTRUCTIONS FOR USE **English**

Note! Other languages available at www.biohithealthcare.com

GastroPanel Four-in-One **REF 606080**

CONTENTS

1. INTENDED USE

GastroPanel Four-in-One test is an *in vitro* microplate-based quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of pepsinogen I (PGI), pepsinogen II (PGII), gastrin-17 (G-17) and IgG antibodies against Helicobacter pylori (Hp) from human EDTA plasma samples. The dedicated software GastroSoft can be used for result interpretation and reporting of all four assays of GastroPanel Four-in-One. Test can be conducted either manually or automatically by the healthcare professionals.

GastroPanel Four-in-One test is intended for diagnosing *H. pylori* infection and atrophic gastritis (AG) from patients with dyspeptic symptoms or at risk to develop malignant cellular changes in stomach mucosa. In addition, the test aids in screening conditions that necessitate additional examination or treatment from healthy stomach mucosa.

2. INTRODUCTION

2.1. Product background

GastroPanel Four-in-One is based on GastroPanel Unified (REF 606400) consisting of GastroPanel Pepsinogen I (REF 606010), GastroPanel Pepsinogen II (REF 606020), GastroPanel Gastrin-17 (REF 606035) and GastroPanel Helicobacter pylori (REF 606040). The basic principle of the test is same and all of the reagents are shared among the two products. Two main differences in GastroPanel Four-in-One compared to GastroPanel Unified are its utilization of singlicate measurements and the use of all four analytes on a single microplate. While GastroPanel Unified uses duplicate measurements and separate plates for each analyte, the performance of GastroPanel Four-in-One is deemed comparable due to set limits within replicate measurements and singlicate measurement accuracy.

2.2. Clinical background

Dyspepsia, defined as predominant epigastric pain that has lasted at least 1 month (1), has an estimated global prevalence of ~20 % (2, 3). The most commonly reported symptoms include epigastric burning, postprandial fullness, early satiety and nausea. Dyspepsia lowers the quality of life and causes economic burden both from societal and health service perspective. (4, 5) Approximately 25 % of dyspepsia patients suffer from gastritis, gastroesophageal reflux disease, peptic ulcer disease or malignancy; the most important organic etiologies of dyspepsia (6). Dyspepsia management in the primary health care vary considerably and often a treatment is determined without a diagnostical test, or gastrointestinal endoscopy and histopathology of biopsies is used to exclude organic origin of condition (7, 8). There are several implications that follow, from overuse of expensive and burdensome endoscopy

to the health issues caused by a long-term use of proton pump inhibitors, or failure to diagnose and treat a *Helicobacter pylori* infection (8–11). *H. pylori* is known to be a root cause for many gastric pathologies, including gastric cancer, gastritis and ulcer, and a chronic infection caused by this pathogen may be associated with various neurological and metabolical disorders, as well as some cardiac and respiratory diseases. Currently, *H. pylori* infection is estimated to affect 50 % of population globally (11, 12). The prevalence of gastric atrophy is approximated to be 5-11 %, varying geographically and in between age-groups, increasing with age and being more common in Asian countries (13). According to Correa cascade (14) model, gastritis is a known risk factor in the establishment of premalignant lesions that may progress to gastric cancer (15–17). Atrophic gastritis also predisposes to malabsorption of vitamin B12, iron, magnesium, zinc, calcium and some medicines.

2.3. Biomarkers

According to different anatomic regions of the stomach, gastric glands differ in morphology as well as in types of specialized cell populations. Corpus and fundus contain simple tubular glands, of which parietal cells and chief cells are responsible for gastric acid and pepsinogen I and II secretion. Antral and pyloric regions contain branched glands, which are composed of endocrine cells secreting gastrins, and mucous cells secreting pepsinogen II. Pepsinogens and gastrins are mainly excreted into the stomach lumen, but a small part diffuses into the blood stream and hence can be measured from a blood sample. The concentrations can be used to evaluate the condition of different regions of stomach mucosa. (18–22)

PEPSINOGENS I AND II

Human gastric mucosa contains aspartic proteinases that can be separated based on their physical properties into two major groups: pepsinogen I (PGI) and pepsinogen II (PGII). Pepsinogens are secreted in the stomach lumen where hydrochloric acid, secreted by the parietal cells, converts them into the corresponding active enzyme pepsins. Pepsinogen synthesis and secretion are regulated by positive and negative feed back mechanisms.

PGI is a precursor enzyme (zymogen) of pepsin, synthesized in the gastric corpus. The circulating PGI concentration closely correlates with the quantity of the chief cells in the corpus mucosa, and any loss of these cells results in decrease in the levels of PGI. As a biomarker, PGI is intended to identify patients who have mucosal atrophy (atrophic gastritis) in the gastric corpus.

Another zymogen, PGII is produced by the chief cells and mucous neck cells of the gastric corpus, in pyloric glands of the gastric antrum, and in Brunner's

glands of the proximal duodenum. Hence, the level of PGII reflects the status of entire gastric mucosa. An elevated PGII level reflects mucosal inflammation, often detected in *H. pylori*-associated non-atrophic gastritis. Since *H. pylori* antibody levels can remain elevated for several months even after successful eradication, PGII is a useful marker for the confirmation of positive eradication results. PGII test complements PGI as an additional diagnostic tool for atrophic corpus gastritis, and PGI/PGII ratio decreases with an increasing grade of atrophy.

GASTRIN-17

Gastrins are linear peptide hormones produced by the G cells in the duodenum, in the pyloric part of the gastric antrum, as well as in the pancreas. The G cells release a mixture of different molecular weight gastrins into the circulation, including gastrin-71, -52, -34, -17, -14, and -6, all of which are carboxy-amidated and circulate in an O-sulfated and non-sulfated form. In healthy humans, the dominant forms of gastrin in plasma are amidated gastrin-34 (G-34) and -17 (G-17), of which G-17 is the predominant and most potent form in healthy antral tissue, and it is almost exclusively produced by the antral G cells. The main function of gastrins is to stimulate the secretion of gastric acid (HCl) by the parietal cells in the gastric corpus, as well as to increase the motility of antrum. In addition, gastrins are known to stimulate gastric chief cells to secrete pepsinogens (PGI, PGII), and induce the contraction of the lower esophageal sphincter.

Amidated form of G-17 included in this test is a direct biomarker of antral structure and function, and through a negative feedback loop, an indirect biomarker of gastric corpus. G-17 plasma levels within the normal range implicate a normal structure and function of the antrum, whereas low or high values of G-17 also reflect abnormal functions of the corpus. The maximum information is obtained when G-17 testing is done separately both for fasting (G-17b) and stimulated (G-17s) levels. The measurement of plasma G-17b may also be used for the monitoring of patients who have undergone gastric surgery; secretion of G-17b is practically zero after successful radical antral resection (antrectomy). In *H. pylori* negative subjects, a low fasting level of G-17 may indicate high acid output.

***HELICOBACTER PYLORI* IgG**

H. pylori is a spiral-shaped, gram-negative bacterium that colonizes in the human stomach. *H. pylori* is the most common cause of chronic infection worldwide, with a prevalence of approximately 50%; however, the majority of the infected individuals are asymptomatic. 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 and may sequel to peptic ulceration and gastric carcinoma (23). IgG immunoglobulins are directed toward several bacterial antigens in an *H. pylori* infection, and these can be detected and quantified from a blood sample.

2.4. GastroPanel®

GastroPanel was developed to meet the need to have a minimally invasive tool for identifying organic origin of dyspepsia symptoms, and to diagnose *H. pylori* infection. The levels of PGI and PGII, G-17 and *H. pylori* antibodies provide information on both the structure and the function of stomach mucosa, hence assisting health care professionals to treat dyspepsia patients and to screen subjects at risk to develop malignant cellular changes (18, 20, 22, 24).

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 (high acid output) and atrophic gastritis in the antrum.

3. PRINCIPLE OF THE TEST

3.1. PGI, PGII, and G-17

The detection of PGI, PGII and G-17 with GastroPanel Four-in-One is based on a sandwich enzyme immunoassay technique with a specific capture antibody adsorbed to a microwell plate, and a detection antibody labelled with horseradish peroxidase (HRP). The assay proceeds according to the following reactions:

1. Human specific monoclonal antibodies attached to a polystyrene surface bind PGI, PGII, and G-17 molecules, respectively, present in the sample.
2. Wells are washed after incubation to remove residual sample.
3. HRP-conjugated secondary detection antibodies are added into the wells, and they bind to specific molecules bound to the capture antibodies in the surface of the wells.
4. The wells are washed, and TMB substrate is added. The substrate is oxidized by the enzyme HRP, resulting in the formation of a blue end product.
5. The enzyme reaction is terminated with the stop solution. The optical density of the developed yellow color is directly related to the PGI, PGII, and G-17 concentration, respectively, of the sample.

3.2. *Helicobacter pylori*

The detection of IgG antibodies against H. pylori with GastroPanel Four-in-One 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. The optical density of the developed yellow color is related to the *H. pylori* antibodies of the sample.

4. TRACEABILITY OF VALUES

As the GastroPanel Four-in-One is based on the GastroPanel Unified, it uses the same calibrators as GastroPanel Unified. The metrological traceability chain of these test calibrators has been established according to the standard ISO 17511:2020. The standard describes methods to establish metrological traceability either to international primary measurement procedures or primary reference materials, and when these are not available, to other calibration materials and methods. As there is no primary material or method for any of the four GastroPanel Four-in-One biomarkers, the reference measurement procedure and calibrators established by the manufacturer were selected as upper end of metrological traceability chain.

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.
CAUTION: Handle blood samples as potentially biohazardous material!

All human blood and control samples are to be treated as potentially infectious and handled according to standard precautions (e.g., GLP, GMMP, CLSI M29). Please refer to internationally or nationally recognized manuals concerning biosafety issues, such as Laboratory Biosafety Manual by World Health Organization or Biosafety in Microbiological and Biomedical Laboratories by Centers for Disease Control and Prevention/National Institutes of Health.

This kit contains reagents manufactured from animal blood or tissue components. All recommended precautions for the handling of bio-derivatives should be followed.

Always use protective gloves and clothing when handling patient samples. Use a safety pipetting device for all liquid transfers. Read all instructions prior to performing this assay.

Components containing ProClin 300 may cause an allergic skin reaction (see Safety Data Sheet). Dispose of ProClin containing solutions according to local waste management legislation.

Any serious incident that occurs in relation to the use of this kit shall be reported immediately to the manufacturer (contact details in chapter 19).

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

The reagents are sufficient for 96 wells, including 24 wells for each of PGI, PGII, G-17, and *H. pylori* test, respectively (pls. see Chapter 6.1. for a detailed description). Reagents of different kit lots must not be mixed.

6.1. Microplate

Contents: 12 x 8 strips in frame coated with high-affinity, monoclonal anti-human-PGI IgG1 antibody, anti-human-PGII IgG1 antibody, anti-human G-17 peptide antibody, and partially purified *H. pylori* bacterial antigen. 3 x 8 strips per analyte per analyte. Strips 1-3 are for H. pylori, 4-6 for PGI, 7-9 for PGII and 10-12 for G-17.
Preparation: Ready for use.
Stability: Stable until expiry date. Discard the strips after use. Component may be used for 6 months after opening. Note that after opening the microplate foil, some crystals may be formed in the bottom of the wells and may affect the results. Discard the crystalized strips.

6.2. Washing Buffer Concentrate (10x)

Contents: 120 ml of 10x phosphate buffer 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 the expiry date. Component may be used for 6 months after opening. The diluted solution is stable for two weeks refrigerated (2 - 8 °C).

6.3. Sample Diluent Buffer

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

Stability: Stable until expiry date. Component may be used for 6 months after opening.

6.4. Substrate Solution

Contents: 15 ml of tetramethylbenzidine (TMB) in aqueous solution.
Preparation: Ready for use.
Stability: Stable until expiry date. Component may be used for 6 months after opening. Avoid exposure to direct light. The substrate solution should be colorless or pale blue/yellow. Any other color indicates deterioration of the substrate solution.

6.5. Stop Solution

Contents: 15 ml of 0.1 mol/l sulfuric acid.
Preparation: Ready for use.
Stability: Stable until expiry date. Component may be used for 6 months after opening.

6.6. Incubation Covers

Three plastic sheets to cover the microplate during incubation.

6.7. Instructions for Use

Inserted into each kit.

6.8. *Helicobacter pylori* specific reagents

- **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 calibrators is printed on the label of the vial.
  Preparation: Ready for use.
  Stability: Stable until expiry date. Component may be used for 6 months after opening.

- **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. Component may be used for 6 months after opening.

- **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. Component may be used for 6 months after opening.

6.9. Gastrin-17 specific reagents

- **Blank solution**
  Contents: One vial containing 1.5 ml of phosphate buffer with 0.1% ProClin 300 as preservative.
  Preparation: Ready for use.
  Stability: Stable until expiry date. Component may be used for 6 months after opening.

- **Calibrators**
  Contents: Four vials, each containing 1.5 ml of gastrin-17 calibrators in phosphate buffer with 0.1% ProClin 300 as preservative. The calibrators have lot-specific G-17 values of approximately 1, 3, 10 and 30 pmol/l. The exact G-17 concentration of the calibrators is labeled on the vials.
  Preparation: Ready for use.
  Stability: Stable until expiry date. Component may be used for 6 months after opening.

- **Control**
  Contents: One vial containing 1.5 ml of control in phosphate buffer with 0.1% ProClin 300 as preservative. The expected gastrin-17 concentration of the control is labeled on the vial.
  Preparation: Ready for use.
  Stability: Stable until expiry date. Component may be used for 6 months after opening.

- **Conjugate**
  Contents: 15 ml of HRP-conjugated anti-human gastrin-17 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. Component may be used for 6 months after opening.

6.10. PGI specific reagents

- **Calibrators**
  Contents: Three vials each containing 1.5 ml of human serum-based calibra- tors with 0.1% ProClin 300 as preservative. The calibrators have lot specific PGI values of approximately 25, 100 and 200 µg/l. The exact PGI concentration of the calibrators is labeled on the vials.

Preparation: Ready for use.
Stability: Stable until expiry date. Component may be used for 6 months after opening.

- **Control**

  Contents: One vial containing 1.5 ml of human serum-based PGI control with 0.1% ProClin 300 as preservative. The expected PGI level of the control is labeled on the vial.
  Preparation: Ready for use.
  Stability: Stable until expiry date. Component may be used for 6 months after opening.

- **Conjugate**

  Contents: 15 ml of HRP-conjugated monoclonal anti-human-PGI in stabilizing buffer with 0.02% methylisothiazolone, 0.02% bromonitrodioxne and 0.002% other active isothiazolones as preservatives.
  Preparation: Ready for use.
  Stability: Stable until expiry date. Component may be used for 6 months after opening.

6.11. PGII specific reagents

- **Calibrators**

  Contents: Three vials each containing 1.5 ml of human serum-based calibrators with 0.1% ProClin 300 as preservative. The calibrators have lot specific PGII values of approximately 6.3, 12.5 and 50 µg/l. The exact PGII concentration of the calibrators is labelled on the vials.
  Preparation: Ready for use.
  Stability: Stable until expiry date. Component may be used for 6 months after opening.

- **Control**

  Contents: One vial containing 1.5 ml of human serum based PGII control with 0.1% ProClin 300 as preservative. The expected PGII level of the control is labelled on the vial.
  Preparation: Ready for use.
  Stability: Stable until expiry date. Component may be used for 6 months after opening.

- **Conjugate**

  Contents: 15 ml of HRP-conjugated monoclonal anti-human-PGII in stabilizing buffer with 0.02% methylisothiazolone, 0.02% bromonitrodioxine and 0.002% other active isothiazolones as preservatives.
  Preparation: Ready for use.
  Stability: Stable until expiry date. Component may be used for 6 months

after opening.

6.12. Blank solution for PGI and PGII

Contents: One vial containing 1.5 ml of human serum-based phosphate buffer with 0.1% ProClin 300 as preservative. Marked as PGI blank solution.
Preparation: Ready for use.
Stability: Stable until expiry date. Component may be used for 6 months after opening.

7. STORAGE AND STABILITY

Store the GastroPanel Four-in-One 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. In use stability for the kit is 6 months. 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 substrate solution should be colorless or pale blue/yellow. Any other color indicates deterioration of the substrate solution.

8. 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 (50µl/1ml plasma; Biohit Oyj, GastroPanel Stabilizer, REF 606050 and REF 606051). 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). Grossly hemolyzed, lipemic or turbid specimens should be discarded.

8.1. 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 and the maximum of two years, the storage should be at –70 °C. Mix the samples thoroughly after thawing. Avoid repeated freezing and thawing of the samples.

8.2. Gastrin-17 stimulation

When a postprandial, protein-stimulated blood sample is needed, a drink made from protein powder (Biohit Oyj, REF 601037 or 601038) 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.

9. MATERIALS REQUIRED BUT NOT PROVIDED

9.1. Manual method

Distilled or deionized water, micropipettes and disposable tips to accurately deliver 20 - 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 (25), e. g. plastic blood collection tube for EDTA plasma, container for ice-water bath, plate shaker.

9.2. Automates

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 (25). Only distilled or deionized water for washing buffer dilution is needed.

10. TEST PROCEDURE

10.1. Preliminary preparations

Allow all reagents and the microplate to reach room temperature (20-25 °C). Dilute the washing buffer concentrate 1 to 10 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 necessary to use calibrators and the control in each test run.

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

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.

Read all instructions and prepare all reagents before starting.

10.2. Manual method

STEP 1: SPECIMEN DILUTION
Dilute the samples as described in figure 1.

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

Figure 1. Sample dilutions.

STEP 2: SAMPLE
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.
Note: The plate or the strips are not color coded. Ensure the correct orientation of the plate before beginning. The plate can be marked to help identify different analyte strips. Strips are analyte specific (Figure 1).

Mix and pipette 100 µl of the blank solution (or dilution buffer), calibrators, controls and diluted samples to the corresponding wells (Figure 1). You may cover the plate with the incubation cover to avoid splashes. Incubate for 60 minutes at ambient temperature with shaking (750 rpm).

Serial dilutions can be used for more convenient sample preparation.

	H.Pylori			PGI			PGII			G-17		
	1	2	3	4	5	6	7	8	9	10	11	12
A	Dil.	S3	S11	BS	S3	S11	BS	S3	S11	BS.	S3	S11
B	Cal1	S4	S12	Cal1	S4	S12	Cal1	S4	S12	Cal1	S4	S12
C	Cal2	S5	S13	Cal2	S5	S13	Cal2	S5	S13	Cal2	S5	S13
D	Cal3	S6	S14	Cal3	S6	S14	Cal3	S6	S14	Cal3	S6	S14
E	Cal4	S7	S15	Ctrl	S7	S15	Ctrl	S7	S15	Cal4	S7	S15
F	Ctrl	S8	S16	-	S8	S16	-	S8	S16	Ctrl	S8	S16
G	S1	S9	S17	S1	S9	S17	S1	S9	S17	S1	S9	S17
H	S2	S10	S18	S2	S10	S18	S2	S10	S18	S2	S10	S18

Figure 2. Recommended pipetting map for the ELISA plate.
Strips are analyte specific as described.

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 analyte 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
Measure the absorbance of microplate wells at 450 nm within 30 minutes. (25)

10.3. Automated method

GastroPanel has been designed 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 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.

11. RESULTS

11.1. 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 Four-in-One is provided with a lot-specific control for each analyte. 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 result must be obtained so that the results can be accepted.

11.2. Calculation of the Results

The absorbance readings are converted to G-17, PGI and PGII concentrations and *H. pylori* IgG immune units (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.

For G-17, PGI and PGII: Subtract the mean OD of the blank (BS) from all OD values of the wells. Plot the OD of the BS (as 0-calibrator) and the calibrators vs. their respective concentration. A second order polynomial fit is adequate to interpolate the unknown concentrations.

A typical calibration curve for Pepsinogen I is shown in Figure 3

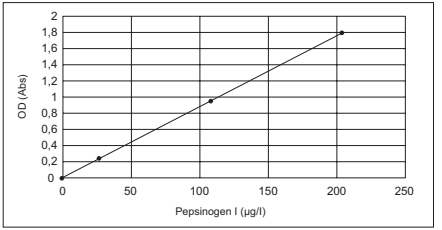

Figure 3. Example of a Typical Calibration Curve for Pepsinogen I.

For *H. pylori* IgG: Subtract the mean OD of the blank from all OD values of the wells. Plot the OD of the calibrators vs. their respective EIU value. Apply logarithmic fit to interpolate the unknown concentrations.

A typical calibration curve for *H. pylori* is shown in Figure 4.

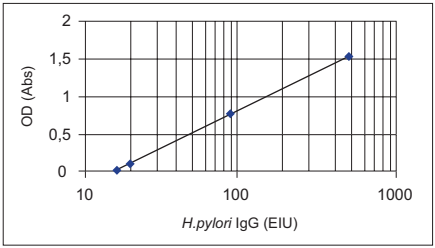

Figure 4. Example of a Typical *H. pylori* Calibrator Curve

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 the use of PPI medication and information about *H. pylori* eradication.

Please refer to section 15 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).

11.3. Interpretation of the Results

- ***Helicobacter pylori* IgG**

  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.

- **Gastrin-17**

  In patients with *H. pylori* infection, a low fasting level of gastrin-17 (<1.0 pmol/l) can be due to two mutually exclusive conditions: 1) the antral mucosa is atrophic (atrophic antrum gastritis), or 2) a high acid output of the stomach (corpus) mucosa. These two conditions can be distinguished by i) gastroscopy, or by ii) using stimulated G-17 of GastroPanel. In antral atrophy, G-17 fails to increase upon protein stimulation, but increases substantially when antral mucosa is intact. The third option to reach the correct diagnosis is to administer a 2-week course of PPI-medication, when G-17 values caused by high acid output will normalize spontaneously, when acid output is suppressed.

- **Pepsinogen I**

  A low plasma PGI level (PGI<30 µg/l) indicates advanced (moderate-to-severe) atrophic gastritis of the corpus. PGI levels below this cut-off are an indication for referral to gastroscopy to confirm the diagnosis and to assess the severity of atrophic gastritis.

- **Pepsinogen II**

  PGII is a marker of mucosal inflammation, most often caused by *Helicobacter pylori* infection or due to prolonged use of PPI medication. A PGI/PGII ratio lower than 3.0 indicates that the patient has advanced corpus atrophy (24, 40).

11.4. Biological Reference Interval

The intervals are based on 7000 Finnish subjects (Biohit internal report, unpublished data).

	Reference range
H. pylori	< 30 EIU
Gastrin-17b	1-7 pmol/l
Gastrin-17s	3-30 pmol/l
Pepsinogen I	30-160 µg/l
Pepsinogen II	3-15 µg/l

12. LIMITATIONS OF THE PROCEDURE

As with any diagnostic procedure, the GastroPanel Four-in-One test results must be interpreted together with the patient's clinical presentation and any other information available to the physician. The NSAIDs may increase the levels of PGII by mucous membrane irritation. Regarding *H. pylori*, 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.

13. ANALYTICAL PERFORMANCE CHARACTERISTICS

All performance tests were carried out at room temperature (20-25 °C). All tests were analyzed with GastroPanel Unified kits and samples were analyzed with duplicate microplate wells. Following chapters refer to GastroPanel Unified kits but describe the performance of GastroPanel Four-in-One as well. Performance is deemed comparable with singlicate measurements due to set limits within replicate measurements.

13.1. Measuring range

For *H. pylori*, repeatability within measurement range (above) has been demonstrated to be ≤ 10 %CV, within-assay precision ≤ 20 %CV and total error at LoQ level ≤ +/- 20%.

For G17, PGI and PGII, within measurement range (above), the method has been demonstrated to be linear within ± 5% nonlinearity bias, repeatability has been demonstrated to be ≤ 8 %CV, within-assay precision ≤ 10 %CV, and total error at LoQ level ≤ ± 20%.

	Lower limit	Upper limit
H. pylori	15 EIU	670 EIU
Gastrin-17	1 pmol/l	30 pmol/l
Pepsinogen I	10 µg/l	200 µg/l
Pepsinogen II	3 µg/l	60 µg/l

13.2. Precision

The precision studies were performed according to the CLSI EP05-A2 guidelines. A panel consisting of seven (*H. pylori* and G17), six (PGI) or four (PGII) EDTA plasma samples over various levels of low, mid and high concentrations of the analyte 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 EP05-A2 guidelines to determine the estimates of repeatability (within-run) precision and within-laboratory precision.

- ***Helicobacter pylori* IgG**

  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 5.4% 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

- **Gastrin-17**

  In the repeatability precision for EDTA plasma samples, the test range was from 1.2 pmol/l to 25.3 pmol/l, the standard deviations from 0.07 pmol/l to 0.74 pmol/l, and the %CV from 2.9% to 5.6%.

  In the Within-Laboratory precision for EDTA plasma samples, the standard deviations range was from 0.11 pmol/l to 1.44 pmol/l, and the %CV from 5.7% to 9.3%.

REPEATABILITY					
Sample	**Mean (pmol/l)**	**%CV**	**Total SD**	**95% CI**	**n**
1	1.2	5.6%	0.07	0.055 to 0.086	76
2	1.6	5.5%	0.09	0.074 to 0.115	80
3	2.1	4.2%	0.09	0.073 to 0.114	80
4	4.9	3.8%	0.18	0.151 to 0.236	80
5	9.2	3.0%	0.28	0.230 to 0.358	78
6	14	3.2%	0.44	0.363 to 0.565	80
7	25.3	2.9%	0.74	0.609 to 0.949	80
WITHIN-LABORATORY					
Sample	**Mean (pmol/l)**	**%CV**	**Total SD**	**95% CI**	**n**
1	1.2	9.3%	0.11	0.094 to 0.137	76
2	1.6	7.2%	0.12	0.101 to 0.142	80
3	2.1	6.2%	0.13	0.111 to 0.158	80
4	4.9	5.7%	0.28	0.237 to 0.341	80
5	9.2	5.6%	0.51	0.431 to 0.635	78
6	14	6.2%	0.87	0.730 to 1.080	80
7	25.3	5.7%	1.44	1.206 to 1.788	80

- **Pepsinogen I**

  In the repeatability precision for EDTA plasma samples, the range for means was from 9.9 µg/l to 182.7 µg/l, the standard deviations from 0.4 µg/l to 6 µg/l, and the %CV from 2.7% to 4.3%.

  In the Within-Laboratory precision for EDTA plasma samples, the range for the standard deviations was from 0.8 µg/l to 12.1 µg/l, and the %CV from 6.6 to 8.5%.

REPEATABILITY					
Sample	**Mean (µg/l)**	**%CV**	**Total SD**	**95% CI SD**	**n**
1	9.9	4.3%	0.43	0.354 to 0.552	80
2	23.0	3.1%	0.70	0.578 to 0.901	80
3	29.4	4.0%	1.17	0.959 to 1.495	80
4	37.0	3.7%	1.36	1.114 to 1.736	80
5	63.8	2.7%	1.75	1.440 to 2.244	80
6	182.7	3.3%	5.95	4.887 to 7.616	80

WITHIN-LABORATORY					
Sample	**Mean (µg/l)**	**%CV**	**Total SD**	**95% CI SD**	**n**
1	9.9	8.4%	0.83	0.697 to 1.029	80
2	23.0	8.0%	1.83	1.521 to 2.307	80
3	29.4	8.0%	2.34	1.960 to 2.909	80
4	37.0	8.2%	3.04	2.529 to 3.813	80
5	63.8	8.5%	5.43	4.482 to 6.878	80
6	182.7	6.6%	12.13	10.057 to 15.287	80

- **Pepsinogen II**

  In the repeatability precision for EDTA plasma samples, the test range was from 2.7 µg/l to 53.5 µg/l, the standard deviations from 0.12 µg/l to 1.56 µg/l, and the %CV from 2.6% to 4.4%.

  In the within-laboratory precision for EDTA plasma samples, the standard deviation range was from 0.22 µg/l to 3.46 µg/l, and the %CV from 6.0% to 7.9%.

REPEATABILITY					
Sample	**Mean (µg/l)**	**%CV**	**Total SD**	**95% CI SD**	**n**
1	2.7	4.4%	0.12	0.098 - 0.153	80
2	6.4	2.7%	0.17	0.138 - 0.216	80
3	12.9	3.4%	0.44	0.364 - 0.567	80
4	34.3	2.6%	0.89	0.732 - 1.142	78
5	53.5	2.9%	1.56	1.284 - 2.001	80
WITHIN-LABORATORY					
Sample	**Mean (µg/l)**	**%CV**	**Total SD**	**95% CI SD**	**n**
1	2.7	7.9%	0.22	0.179 - 0.269	80
2	6.4	6.2%	0.40	0.329 - 0.499	80
3	12.9	6.7%	0.87	0.726 - 1.074	80
4	34.3	6.0%	2.07	1.718 - 2.610	78
5	53.5	6.5%	3.46	2.821 - 4.475	80

13.3. Linearity

The linearity of GastroPanel Gastrin-17, Pepsinogen I and Pepsinogen II was determined in accordance with the CLSI Guideline EP06-A. Three kit lots were tested. A logarithmic transformation of the data was used to correct the data set to be closer to Gaussian distribution. The method has been demonstrated to be linear within +/- 5% nonlinearity bias at this interval.

	Lower limit of linearity	Upper limit of linearity
Gastrin-17	0.9 pmol/l	31.4 pmol/l
Pepsinogen I	10.2 µg/l	199.2 µg/l
Pepsinogen II	3.2 µg/l	60.1 µg/l

13.4. Detection limit and quantitation limit

The limit of blank (LoB) and the limit of detection (LoD) was determined consistent with the CLSI Guideline EP17-A 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. Three (G17), four (*H. pylori*) or five (PGI and PGII) EDTA plasma samples and three kit lots were used to establish the LoD and kit diluent buffer for establishing the LoB.

The limit of quantitation was determined consistent with the NCCLS (*H. pylori*) or CLSI (G17, PGI and PGII) Guideline EP17-A based on 60 determinations of three (G17), four (*H. pylori*) or five (PGI and PGII) 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. Total error and %CV was defined for the LoQ.

	LoB	LoD	LoQ	Total error	%CV
H. pylori	13.1 EIU	14.7 EIU	15.0 EIU	-10.7%	5.2%
G-17	0.2 pmol/l	0.4 pmol/l	1.1 pmol/l	-17.8%	9.3%
PGI	0.9 µg/l	1.5 µg/l	8.7 µg/l	-15.6%	7.8%
PGII	0.2 µg/l	0.4 µg/l	1.9 µg/l	-11.1%	6.1%

13.5. Analytical specificity

As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse (HAMA) or heterophilic antibodies in the sample. Patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA) and may show either falsely elevated or depressed values when tested. This applies to all the analytes below.

- **Gastrin-17**

  The GastroPanel Gastrin-17 was evaluated for cross-reaction by related peptides gastrin-34, gastrin-13 and cholecystokinin (CCK) by spiking two samples at Gastrin-17 levels of approximately 1.7 pmol/l and 14 pmol/l. A bias caused by 200 pmol/l gastrin-34, gastrin-13 or CCK was less than +/- 3.5%. This was not considered a significant bias.

- **Pepsinogen I**

  The GastroPanel Pepsinogen I was evaluated for cross-reaction by pepsinogen II by spiking two samples at pepsinogen I levels of approximately 30 µg/l and 100 µg/l. A bias caused by 100 µg/l pepsinogen II was less than 6% (2.4% and 5.5 %, respectively). This was not considered as a significant bias.

- **Pepsinogen II**

  GastroPanel Pepsinogen II was evaluated for cross-reaction by pepsinogen I by spiking two samples at pepsinogen II levels of approximately 2.8 µg/l and 13 µg/l. A bias caused by 400 µg/l pepsinogen I was less than +/- 4% (-3.5 % and 2.2%, respectively). This was not considered as a significant bias.

13.6. Interference

Interference was evaluated according to CLSI Guideline EP07-A2.

- ***Helicobacter pylori* IgG**

  The bias caused by hemoglobin, unconjugated bilirubin, conjugated bilirubin and triglycerides at concentrations of 2 g/l, 15 mg/dl, 5 mg/dl and 500 mg/dll, 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.

- **Gastrin-17**

  The bias caused by hemoglobin, unconjugated bilirubin, conjugated bilirubin and triglycerides at concentrations of 2 g/l, 15 mg/dl, 5 mg/dl and 500 mg/dl, respectively, was found to be less than 10% at Gastrin-17 plasma levels of approximately 1.5 pmol/l and 13 pmol/l. This was considered to be a non-significant interference. Grossly hemolyzed, lipemic, or turbid specimens should be avoided.

- **Pepsinogen I**

  The bias caused by hemoglobin, unconjugated bilirubin, conjugated bilirubin or triglycerides at concentrations of 2 g/l, 15 mg/dl, 5 mg/dl and 500 mg/dl, respectively, was found to be less than 10% at PGI plasma

levels of 31 µg/l and 100 µg/l. This was considered as a non-significant interference. Grossly hemolyzed, lipemic, or turbid specimens should be avoided.

- **Pepsinogen II**

  The bias caused by hemoglobin, unconjugated bilirubin, conjugated bilirubin or triglycerides at concentrations of 2 g/l, 15 mg/dl, 5 mg/dl and 500 mg/dl, respectively, was found to be less than 10% at plasma levels of approximately 2.8 µg/l and 12 µg/l. This was considered as a non-significant interference.

14. DIAGNOSTIC PERFORMANCE

14.1. *Helicobacter pylori*

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.

14.2. Gastrin-17

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.

Concordance* between the mean values of the biomarkers in the standard (REF 601035) and the GastroPanel Gastrin-17 test (REF 606035).

GastroPanel test version	G-17b (M±SD)	G-17s (M±SD)
Gastrin-17 (REF 601035)	7.7 (12.0)	17.6 (16.5)
GastroPanel Gastrin-17 (REF 606035)	6.9 (9.5)	17.0 (14.2)
ICC**	0.982 (0.971-0.988)	0.984 (0.964-0.992)
Correlation	0.969	0.978

*Calculated by intra-class correlation coefficient (ICC; weighted kappa) and Pearson bivariate correlation tests

**ICC under most stringent conditions (strict parallel two-way random model, absolute agreement, average measures settings).

14.3. Pepsinogen I

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.

Concordance* between the mean values of the biomarkers in the standard pepsinogen I (REF 601010.01) and the GastroPanel Pepsinogen I test (REF 606010).

GastroPanel test version	PGI (M±SD)
Pepsinogen I (REF 601010.01)	102,9 µg/l (47,4)
GastroPanel Pepsinogen I (REF 606010)	89.2 µg/l (42.5)
ICC**	0.966 (0.409-0.990)
Correlation	0.983

*Calculated by intra-class correlation coefficient (ICC; weighted kappa) and Pearson bivariate correlation tests

**ICC under most stringent conditions (strict parallel two-way random model, absolute agreement, average measures settings).

14.4. Pepsinogen II

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.

Concordance* between the mean values of the biomarkers in the standard Pepsinogen II (REF 601020.02) and the GastroPanel Pepsinogen II test (REF 606020).

GastroPanel test version	PGII (M±SD)	PGI/PGII (M±SD)
Pepsinogen II (REF 601020.02)	11.2 (8.4)	11.3 (5.2)
GastroPanel Pepsinogen II (REF 606020)	15.2 (10.1)	6.8 (2.7)
ICC**	0.937 (0.084-0.983)	0.877(0.818-0.917)#
Correlation	0.981	0.952

*Calculated by intra-class correlation coefficient (ICC; weighted kappa) and Pearson bivariate correlation tests; **ICC under most stringent conditions (strict parallel two-way random model, absolute agreement, average measures settings); # ICC using parallel two-way random model with consistency and average measures settings.

15. 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) (26–28). 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.

15.1. 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 (18, 29–32). 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.

15.2. 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 (18, 21, 29, 33). 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 2 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 REF 601038).

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

The regulation above also works in the other way around. 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 (29, 33). 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 PPI medication. In that case, the antral G-17b should be normalized within two weeks (17.8.).

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

Like all bacteria, *Helicobacter pylori* will also induce acute inflammation in the gastric mucosa, with the usual onset in the antrum (18, 26, 29, 34–36). Three different marker profiles can be encountered in association with *Hp* infection.

15.4a 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 (29, 34, 36).).

15.4b 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).

15.4c In cases where *Hp* eradication attempt fails, *Hp* antibody levels remain elevated (usually slightly) and PGI and PGI/PGII ratio usually fall 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 (29, 36).

15.5. 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 a reduced PGI/PGII ratio, which is another excellent signature of AG in the corpus (18, 19, 21, 29, 30, 34, 37–39). This reduction in the 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 (32, 33). 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.

15.6. 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 (21, 33, 40).

15.7. 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 (18, 19, 21, 29, 30, 32–34, 37–39). 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 level below the 30 EIU cut-off level.

15.8. 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 produc- tion 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. (18, 29, 31, 33)

* 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*.

Table 1. The eight diagnostic categories of GastroPanel (*see previous page for explanation)

	GastroPanel Biomarkers						Interpretation
	Pepsinogen I (30-160 µg/l)@	Pepsinogen II (3-15 µg/l)	PGI/ PGII ratio (3–20)	Gastrin-17b (1–7 mol/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 successfullyn 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

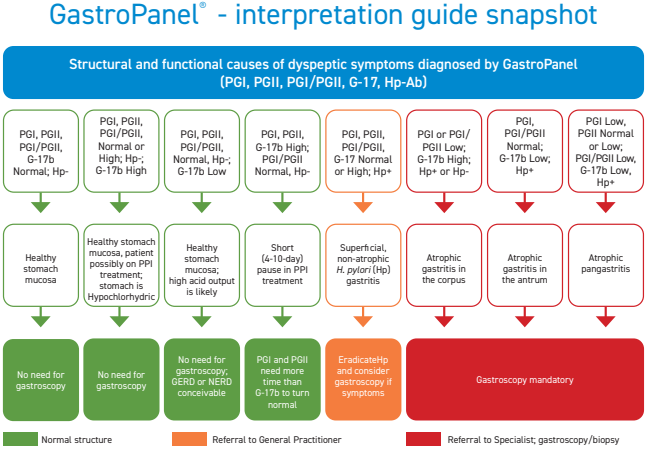

Figure 5. GastroSoft interpretation guide

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 the diagnosis of dyspepsia and *H. pylori* infection, e.g., 13C-urea breath test (UBT) stool antigen test, or antibody test. In subjects with AG, MALT lymphoma, or bleeding peptic ulcer, and in those on PPI medication or antibiotics, the UBT or stool antigen tests frequently give false negative results, and *H. pylori* infection (with all its risks) remains undetected (28, 36, 41–43) (www.biohithealthcare.com/additional-information).

GastroPanel is capable of diagnosing atrophic gastritis affecting either the corpus or antrum or both. As 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, the mucosal atrophy (mild atrophy in particular) is a subjective diagnosis, with substantial interobserver variation among pathologists. Similarly, the accuracy of gastroscopy is dependent on the experience and competence of the gastroscopist. GastroPanel is devoid of these shortcomings, because it is an automated ELISA-based laboratory assay. In fact, endoscopic biopsy histology is not a reliable gold standard (46), albeit currently used as such. As compared with serum biomarkers, its limitations in diagnostic accuracy should be kept in mind (24, 47).

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 (21). Importantly, the diagnosis of gastric atrophy is highly subjective without use of gastric biopsies, i.e., on the basis of gastroscopy alone (48). 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.

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17. DATE OF ISSUE

GastroPanel Four-in-One kit insert.

Version 1.0, April 2022.

18. 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.

This Biohit diagnostic kit has been manufactured according to ISO 9001/ISO 13485 quality management protocols and has passed all relevant Quality Assurance procedures related to this product.

In case of any serious incident in relation to the product, contact the manufacturer.

19. ORDERING INFORMATION

GastroPanel Four-in-One

REF 606080

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NOTES

20. 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 blank solutions (G-17, and PGI, PGII) or dilution buffer (*H. pylori*), the calibrators, the control, and diluted (1:400 for *H. pylori*, 1:20 for PGI and PGII, 1:5 for G-17) patient samples into the wells within 20 minutes.

*

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

*

Wash the wells 3 times with 350 µl of the diluted washing buffer

*

Pipette 100 µl of the analyte specific conjugate solutions into the wells

*

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

*

Wash the wells 3 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 avoiding direct light

*

Pipette 100 µl of the mixed stop solution into the wells

*

Read at 450 nm within 30 minutes