BIOHIT
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GastroPanel® Pepsinogen II

ELISA kit for the measurement of human pepsinogen II in EDTA plasma as part of GastroPanel

INSTRUCTIONS FOR USE

GastroPanel®
Product Family
606400

REF 606020

IVD CE

For *in vitro* diagnostic use
Store at 2-8°C upon receipt

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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 to 8°C
	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
BLANK	Blank solution

INSTRUCTIONS FOR USE **English**

Note! Other languages available at www.biohithealthcare.com

GastroPanel® Pepsinogen II **REF 606020**

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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. pylor*-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 antifungal 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. INTENDED USE

GastroPanel Pepsinogen II (PGII) test is an *in vitro* microplate-based quantitative enzyme-linked immunosorbent assay (ELISA) for the determination of human pepsinogen II from EDTA plasma samples and is intended to be used in conjunction with the other tests of GastroPanel Unified (REF 606400): GastroPanel Pepsinogen I (REF 606010), GastroPanel Gastrin-17 (REF 606035), and GastroPanel *Helicobacter pylori* (REF 606040). The dedicated software GastroSoft can be used for result interpretation and reporting of all four tests of GastroPanel Unified. Test can be conducted either manually or automatically by the healthcare professionals.

GastroPanel Unified 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, GastroPanel Unified test aids in screening conditions that necessitate additional examination or treatment from healthy stomach mucosa.

The level of pepsinogen II (PGII) biomarker reflects the status of entire gastric mucosa. Thus, the PGII test complements the GastroPanel Pepsinogen I (PGI) test (REF 606010) to aid in diagnosis of atrophic corpus gastritis. In addition, the PGII test is used for screening of mucosal inflammation and monitoring the successful eradication of *H. pylori*.

3. PEPSINOGEN II BACKGROUND

Pepsinogen II 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. The ratio of pepsinogen I (PGI) to PGII plasma levels in normal subjects is about 3-20 [9].

The PGI/PGII ratio decreases linearly with increasing grade of atrophic gastritis

in the corpus [24, 25]. The ratio falls below 3.0 when atrophic corpus gastritis is advanced (moderate or severe) [25]. It has been shown that the risk of gastric cancer is increased (5-fold) when the PGI/PGII ratio is low [1, 8, 27-35]. This test is intended as an additional diagnostic tool for atrophic corpus gastritis, which is a known risk condition for gastric cancer [24, 26]. The Pepsinogen II assay is designed for use concomitantly with the Pepsinogen I assay to determine the PGI/PGII ratio, alongside Gastrin-17 to confirm the diagnosis of atrophic gastritis of the corpus (G-17 is up-regulated). An elevated PGII level reflects mucosal inflammation, the highest values being detected in *Hp*–associated non-AG. Since *Hp* antibody levels can remain elevated for several months, even after successful eradication, PGII is a useful marker for the confirmation of positive eradication results.

4. PRINCIPLE OF THE TEST

This GastroPanel PGII is based on a sandwich enzyme immunoassay technique with a PGII-specific capture antibody adsorbed on a microwell plate and a secondary detection antibody labelled with horseradish peroxidase (HRP).

The assay proceeds according to the following reactions:

1. Human PGII-specific monoclonal antibodies attached to a polystyrene surface bind PGII molecules 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 PGII molecules bound to PGII 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 PGII concentration of the sample.

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

CAUTION: Handle the plasma samples as potentially 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, 2009, 5th ed. (CDC/NIH) and No. (CDC) 21-1112 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 a blood derivative should be followed.

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.

6. TRACEABILITY OF VALUES

There is no international reference material to pepsinogen II. The pepsinogen II calibrator and control values are assigned to Biohit internal master calibrators.

7. 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.

7.1. Microplate

Contents: 12 x 8 strips in a frame coated with high-affinity, monoclonal anti-human-PGII IgG1.

Preparation: Ready for use. Do not combine of strips/wells from different kits of the same lot.

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.

7.2. Washing Buffer Concentrate (10x)

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

7.3. Sample Diluent Buffer

Contents: 50 ml 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.

7.4. Blank Solution

Contents: One vial containing 1.5 ml of human serum-based 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.

7.5. 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.

7.6. 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.

7.7. 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.

7.8. 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.

7.9. Stop Solution

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

Preparation: Ready for use.

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

7.10. Incubation Covers

Three plastic sheets to cover the microplate during incubation.

7.11. Instructions for Use

Inserted into each kit.

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 (50 µl/1 ml plasma; Biohit Oyj, GastroPanel Stabilizer, REF Nos. 606050 and 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 (20) 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 15 - 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 [36], e.g., plastic blood collection tube for plasma, container for ice-water bath, plate shaker.

9.2 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 [36].

10. STORAGE AND STABILITY

Store the GastroPanel Pepsinogen II kit refrigerated (2-8°C). When stored at these temperatures, the kit is stable until the expiration date indicated 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 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 any of the 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.

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

11.1. 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 PGI and PGII, Figures 2 and 3 for G-17 and *H. pylori*, 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	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 1. Pipetting order of PGI and PGII

	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	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 3. Pipetting order of H. pylori

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 [36].

11.2. 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.

12. RESULTS

12.1. Quality Control Values

Good Laboratory Practice requires the use of appropriate controls to establish that all the reagents and protocols are performing as designated. GastroPanel Pepsinogen II is provided with a lot-specific 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.

12.2. Calculation of the Results

The absorbance readings are converted to PGII concentrations 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 (BS) from all OD values of the wells. Plot the mean 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 is shown in Figure 4.

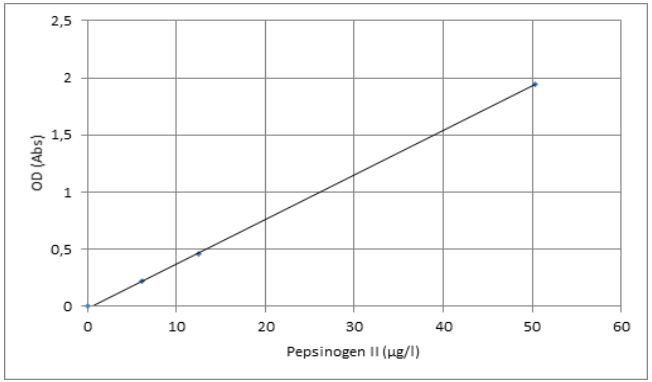


Figure 4. A typical calibration curve.

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 software applications and services. More information is also available on the GastroPanel product site (www.gastropanel.com).

12.3. Interpretation of the results

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 (moderate-to-severe) corpus atrophy [15, 2].

12.4. Biological reference interval

The reference range is 3-15 µg/l. The interval is based on 7000 Finnish subjects (Biohit internal report, unpublished data). It is recommended that the reference values are considered as guidelines only.

13. LIMITATIONS OF THE PROCEDURE

As with any diagnostic procedure, GastroPanel Pepsinogen II 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.

14. 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 Pepsinogen II is from 3 µg/l to 60 µg/l.

In this range, 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%.

Precision:

The precision studies were performed according to the CLSI EP5-A2 guidelines. A panel consisting of five EDTA plasma samples over various levels of low, mid and high pepsinogen II concentrations 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 and within-laboratory precision.

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

Linearity:

The linearity of GastroPanel Pepsinogen II was determined consistent with the CLSI Guideline EP06-A. Three kit lots were tested. A logarithmic transformation of the data was used to correct the data set closer to Gaussian distribution.

The method has been demonstrated to be linear from 3.2 µg/l to 60.1 µg/l within +/- 5% nonlinearity bias at this interval.

Detection limit and quantitation limit:

The limit of blank (LoB) and the limit of detection (LoD) for GastroPanel Pepsinogen II 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 blank and 60 low-level samples. Five EDTA plasma samples and three kit lots were used.

The LoB was found to be 0.2 µg/l and the LoD 0.4 µg/l.

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

The LoQ was found to be 1.9 µg/l with a total error of 11.1% and with a CV% between measurements of 6.1%.

Analytical specificity:

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.

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.

Interference:

GastroPanel Pepsinogen II was evaluated for interference according to CLSI Guideline EP07-A2. The bias caused by hemoglobin, conjugated bilirubin, unconjugated bilirubin or 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 plasma levels of approximately 2.8 µg/l and 12 µg/l. This was considered as a non-significant interference.

15. 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.

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.

16. 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 (pangastritis)[13, 37, 38]. 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.

16.1 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.

16.2 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 REF No. 601038).

16.3. 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 [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].

16.4. 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, 39]. Three different marker profiles can be encountered in association with *Hp* infection.

16.4a 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, 39].

16.4b 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 [39].

16.4c 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 [3, 39].

16.5 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.

16.6 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].

16.7 Atrophic gastritis of the antrum and corpus

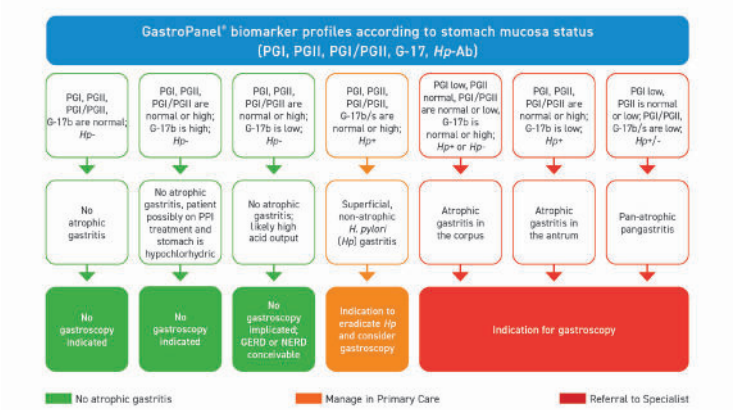
The most severe form of AG is known as atrophic pan-gastritis, affecting both the antrum and corpus. As an end result, the specified cells (chief cells) in the corpus and antrum (G cells) disappear, leading to a biomarker expression pattern where both pepsinogens (PGI, PGII) and G-17 are substantially 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 (16.5), *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 below the 30 EIU cut-off level.

16.8 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]

GastroPanel® - interpretation guide snapshot



Abbreviations: PGI = Pepsinogen I; PGII = Pepsinogen II; G-17b = basal/fasting Gastrin-17; G-17s = stimulated/postprandial Gastrin-17; *Hp*+ = *H. pylori*–positive (infected); *Hp*– = *H. pylori*–negative (not infected). Normal = within reference range; High = above reference range; Low = below reference range.

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 [40-44].

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 [45], although it is currently used as such. Compared with serum biomarkers, its limitations in diagnostic accuracy should be kept in mind [2, 46].

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 [47]. 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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18. DATE OF ISSUE

GastroPanel Pepsinogen II kit insert.
Version 8.0, 2026-04-16

19. 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 13485 quality management protocols and have passed all relevant Quality Assurance procedures related to these products.

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

20. ORDERING INFORMATION

GastroPanel®
REF No. 606400.

21. 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 solution, the calibrators, the control, and diluted (1 to 20) patient samples into the wells

*

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

*

Wash 3 times with 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 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

*

Pipette 100 µl of the mixed stop solution into the wells

*

Read at **450 nm** within 30 minutes

NOTES

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