ANTICANCER RESEARCH *39*: 1091-1104 (2019)
doi:10.21873/anticanres.13218

*Review*

# GastroPanel® Biomarker Assay: The Most Comprehensive Test for *Helicobacter pylori* Infection and Its Clinical Sequelae. A Critical Review

KARI SYRJÄNEN[1,2], MATTI ESKELINEN[3], ANTS PEETSALU[4], TOOMAS SILLAKIVI[4], PENTTI SIPPONEN[5], MATTI HÄRKÖNEN[1], LEA PALOHEIMO[6], MINNA MÄKI[6], TAPANI TIUSANEN[6], OSMO SUOVANIEMI[1,6], FRANCESCO DiMARIO[7] and ZHU PING FAN[8]

[1]*Department of Clinical Research, Biohit Oyj, Helsinki, Finland;*
[2]*Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, Brazil;*
[3]*Department of Surgery, Kuopio University Hospital, Kuopio, Finland;*
[4]*Department of Surgery, University of Tartu, Tartu, Estonia;*
[5]*Patolab Oy, Espoo, Finland;*
[6]*Department of Research and Development, Biohit Oyj, Helsinki, Finland;*
[7]*Department of Clinical and Experimental Medicine, University of Parma, Parma, Italy;*
[8]*Shanghai Jiaotong University School of Medicine, Shanghai Institute of Digestive Disease, Shanghai, P.R. China*

**Abstract.** *Background/Aim: Several clinical conditions seriously hamper the diagnostic accuracy of the commonly used tests for Helicobacter pylori (Hp), $^{13}$C-urea breath test (UBT) and stool antigen test (SAT). The present communication is a critical review of the potential limitations of UBT and SAT, and describes the approach on how these can be avoided. Drawbacks of the Hp tests: False-negative results are most often due to low bacterial load in the stomach due to: i) use of proton pump inhibitor medication; ii) use of antibiotics; iii) presence of atrophic gastritis and hypoacid stomach; iv); bleeding peptic ulcer; v) gastric cancer (GC) and vi) mucosal-associated lymphatic tissue lymphoma. The UBT also gives false-positive results when urease-producing bacterial species, other than Hp colonize an acid-free stomach. Importantly, neither UBT nor SAT are capable of diagnosing atrophic gastritis, thus missing the patients at highest risk for GC. GastroPanel® (Biohit Oyj, Finland) circumvents these shortcomings with a serological test consisting of a panel of stomach-specific biomarkers: pepsinogen I, pepsinogen II, gastrin-17 and Hp antibodies. GastroPanel® is a tool for non-invasive examination of i) dyspeptic patients for exclusion or diagnosis of Hp or atrophic gastritis, also disclosing the status of gastric acid output; ii) for screening of asymptomatic individuals at risk of GC; and iii) for comprehensive diagnosis of Hp infection. GastroSoft® application integrates the biomarker profile with the patient's medical information, accurately classifying the biomarker profiles into eight diagnostic categories. Conclusion: Given that Hp is the single most important risk factor of GC, the non-invasive diagnosis and screening of Hp should be based on more accurate and more comprehensive testing than UBT or SAT alone. The GastroPanel® is such test, being completely devoid of the known serious shortcomings of UBT and SAT.*



*Correspondence to:* Professor Kari Syrjänen, MD, Ph.D., FIAC; Department of Clinical Research, Biohit Oyj, Laippatie 1, FIN-00880 Helsinki, Finland. Tel: +358 405566810, e-mail: kasyrja@netti.fi

*Key Words: Helicobacter pylori*, diagnosis, $^{13}$C-urea breath test, stool antigen test, diagnostic errors, false-negative, false-positive, limitations, serum biomarker panel, GastroPanel®, pepsinogen I, pepsinogen II, gastrin-17, GastroSoft®, gastric cancer, risk, atrophic gastritis, acid output, review.

The understanding of the important role of *Helicobacter pylori* (Hp) infection in the pathogenesis of gastric cancer (GC) and peptic ulcer disease has increased progressively since the discovery of this bacterium in 1984 by Marshall and Warren (1). According to the current concepts, GC develops in individuals with Hp infection through precursor lesions of progressively increasing severity from non-atrophic Hp associated gastritis to mild, moderate and severe atrophic gastritis (AG), accompanied by intestinal metaplasia

(IM) and dysplasia. This sequence of events is known as the Correa cascade and is estimated to be involved in around 50% of GC cases, the intestinal type in particular (2-4).

In parallel with an increased understanding of the pathogenetic mechanisms, the management of Hp infection has also undergone remarkable development during the past decades. Much of this development can be attributed to the European *Helicobacter* Study Group that took its first initiative in 1996 in Maastricht to gather dedicated experts in the field to review and discuss the pertinent clinical data to create recommendations for the clinical management and diagnosis of Hp infection (5). Since then, these Maastricht conferences have been repeated every 4-5 years, each being followed by a Consensus Report, the latest (2017) being the fifth (6). Attempts to standardize the diagnosis and treatment of Hp infections within countries have led to several national guidelines as well (7, 8). In all these reports, considerable attention has been paid to different diagnostic methods available for Hp detection, also including comprehensive review of the advantages and limitations of each technique and their utility in different settings (6-10).

As is frequently the case in daily practice, there is a common tendency to overlook the limitations of the commonly used Hp tests, although their shortcoming has been clearly discussed in all European Consensus Reports since 1996 (5, 6, 9, 10). This applies to both of the two most widely used Hp tests: the $^{13}$C-Urea Breath Test (UBT) and Stool Antigen test (SAT), for which Barry Marshall who discovered Hp with Robin Warren (1) made an early warning already some 20 years ago (11). Based on the substantial literature accumulated during the past two decades, there is little doubt that several clinical conditions seriously hamper the diagnostic value of these two Hp tests, false-negative (up to 40%) and false-positive results (UBT) are not uncommon in both (12, 13).

The present communication has a dual aim: i) it is a critical review of the known limitations of the UBT and SAT in diagnosis of Hp infection; and ii) it introduces the reader to a novel approach as to how these downsides of UBT and SAT can be avoided in clinical practice.

## The UBT

The UBT is based on the ability of Hp to break urea down into carbon dioxide, which then is absorbed from the stomach and eliminated in the breath (5-10). For the UBT, patients swallow a capsule containing urea made from an isotope of carbon ($^{13}$C). If Hp is present in the stomach, the urea is turned into carbon dioxide. Breath samples are collected and $^{13}$C in the exhaled carbon dioxide is measured. If the isotope is detected in the breath, it means that Hp is present in the stomach. Basically, the $^{13}$C signal in breath is directly dependent on the level of urease activity in stomach juice, *i.e.* on the Hp bacterial load.

Both recent national and European Consensus present data on the utility of UBT in specific diagnostic settings (5, 6). The use and limitations of UBT were exhaustively discussed at an Italian conference in Bologna (February 2015), where recommendations were based on the best current evidence to help physicians manage Hp infection in Italy. The guidelines have been endorsed by the Italian Society of Gastroenterology and the Italian Society of Digestive Endoscopy (8). The same topics were also surveyed in the latest Maastricht Consensus Conference (6).

The Italian Consensus report stated that: "several meta-analyses confirm that UBT is the best test for non-invasive Hp diagnosis, with a 96% sensitivity and a 93% specificity" (8). On closer examination, however, this statement refers to one meta-analysis only (13), which included only cross-sectional studies evaluating the diagnostic accuracy of UBT in adult patients with dyspeptic symptoms, making the meta-analysis highly biased. Thus, out of 1,380 studies identified in the literature, only 23 met the authors' eligibility criteria (13). The meta-analysis was associated with a significant statistical heterogeneity that remained unexplained after subgroup analysis. The included studies also had a moderate risk of bias. The authors concluded that UBT has a high diagnostic accuracy for detecting Hp infection in patients with dyspepsia. They admit, however, that the reliability of their meta-analytic estimates is limited by significant between-study heterogeneity (13).

*False-negative results*. The UBT has serious limitations confined to special situations where the test results should be interpreted with caution (5-13). There is firm evidence to indicate that recent (within 2 weeks) use of proton pump inhibitors (PPI) or antimicrobials (within 4 weeks) may lead to a decrease in the gastric bacterial load causing false-negative results (14-16). Gastric bleeding can also reduce the sensitivity of both UBT and SAT (14, 15). Data from a systematic review suggested repeating the diagnostic tests in patients with bleeding ulcer after at least 4 weeks in the case of a negative UBT result (17). In patients with precancerous conditions (*e.g.* AG, IM) or GC, as well as in patients with partial gastrectomy, diagnostic tests may have lower accuracy (3, 17).

The same limitations as acknowledged in the Italian Consensus Report (8) are also emphasized as limitations of the UBT and SAT tests in the latest Maastricht Consensus Report (6). It is clearly stated that significant decrease of the gastric Hp load arises from the following conditions: i) use of antimicrobial agents, ii) use of anti-secretory drugs (PPI), and iii) in bleeding ulcers. Importantly, bacterial load may be permanently low in premalignant and malignant lesions, including i) AG, ii) IM, or iii) mucosal-associated lymphatic tissue (MALT) lymphoma (18, 19).

*PPI medication*. Several studies have shown that by increasing the gastric pH, PPI use leads to local changes in the gastric environment and load of Hp in the stomach (14). Because PPI drugs have antimicrobial properties, while converting the stomach into an alkaline and hostile environment for Hp growth, the bacterial load decreases, especially in the antrum, contributing easily to false-negative results of the UBT, in contrast to serology (Hp IgG or IgA antibodies), which remains unaffected in those taking PPI medication. Most of these studies in patients taking PPIs have been carried out with UBT and showed a 10-40% false-negative rate (14, 20). In addition to PPI treatment (14-16), $H_2$-blockers may also give false-negative UBT results, albeit to a lesser extent than PPI medication (12, 21).

*Antimicrobials*. The evidence is notwithstanding that the local Hp load in the stomach will be reduced by the use of antimicrobials, leading to potentially false-negative UBT results, as clearly stated in the Consensus Reports (5, 8, 14-16, 22, 23). Thus, Perri *et al*. (22) performed UBT in 41 Hp-infected individuals before and 1 day after therapy with amoxycillin (2.5 g). They showed that even a short course of antibiotics specific for Hp may result in a false-negative UBT (22). The authors concluded that false-negative results are likely even after 1 day of therapy with bactericidal or even with anti-secretory (PPI) drugs. In another recent study, Leung *et al*. examined the serial changes of UBT results in 35 hospitalized patients who were given antibacterial therapy for chest or urinary infections (23), most (91%) receiving a single antibiotic (penicillin or cephalosporin group). Serial UBTs were performed within 24 hours of antibiotics onset, and at 1- and 6-weeks post-therapy. The results showed that one-third of Hp-infected individuals had transient false-negative UBT results during their treatment, although a full clearance of Hp infection by regular antibiotic consumption was very rare (23).

*Bleeding ulcer.* As to the accuracy of UBT in cases of bleeding ulcers, this topic was recently subjected to a comprehensive meta-analysis (17). It has been suggested that prevalence of Hp in peptic ulcer bleeding is lower than that in those with non-complicated ulcers. In a systematic review of the studies assessing the prevalence of Hp infection in patients with peptic ulcer bleeding, including 71 articles and 8,496 tested patients, UBT was reliable only when delayed until at least 4 weeks after the episode of peptic ulcer bleeding (17, 24, 25).

*Cancer precursors: AG and acid-free stomach*. AG is another clinical condition associated with a substantial proportion of false-negative UBT results, particularly in patients with AG with a chronically hypo-acid or acid-free stomach. This topic has been studied in detail by Kokkola *et al*. (19, 26, 27). In their first study, patients with atrophic corpus gastritis (AGC) and elevated Hp antibody level, but with Hp-negative UBT and histology, were randomized into eradication therapy and follow-up only (19). Hp antibody level decreased significantly in 6/7 patients in the eradication group, while in the control group, the antibody level declined in only 1/8 patients. Thus, in patients with AGC, a positive Hp serology may indicate an ongoing infection in spite of negative UBT and histology.

In another study, these authors made a direct comparison of UBT, Hp serology and histology in 50 male patients with histologically verified AGC (26). The results are revealing: Hp was detected in 15 (30%) patients by histology and in 14 (28%) by UBT, whereas increased serum Hp antibody levels were found in 41 (82%) patients ($p<0.0001$). Altogether, Hp infection was associated with AGC in 84% of the patients. The authors concluded that in patients with AG and IM, prevalence of Hp infection will be underestimated if only UBT is relied on (26).

Similar conclusions were drawn in another recent study by Lahner *et al*. (28), who examined 27 patients with AGC using UBT and SAT to assess whether the diagnostic yield of Hp in AGC would be higher by these two tests than with histology alone. The results showed that in patients with AGC, neither the UBT nor SAT added any useful information regarding Hp infection, but a combination of histology and serology was needed to define the Hp status of the patients (28). However, when followed-up for long enough, Hp antibodies also disappeared spontaneously within 10 years in almost a quarter of the patients with advanced AGC. This disappearance of Hp antibodies is accompanied by only a mild or no improvement in the gastric mucosal status (27). Thus, an Hp test can give a negative result in AGC, due either to i) the disappearance of Hp during the protracted course of the disease (27, 28), or ii) because AGC is caused by an autoimmune disease and not Hp (2, 4).

*MALT lymphoma*. MALT lymphoma is another specific condition ascribed to Hp infection (6, 8), but known to be associated with a low bacterial load, making it susceptible to false-negative UBT results (5, 6, 8-10). Because gastric MALT lymphoma is a rare disease, few studies comparing the accuracy of diagnostic tests in these patients have been published, and only a limited number of tests have been compared (18). In one of those few studies, a total of 90 patients with low-grade gastric MALT lymphoma were enrolled, comparing histology, serology polymerase chain reaction and culture. Histology (97.5%) and serology (95.0%) were the two most sensitive tests, far superior to the other Hp tests (18).

Although patients with gastric MALT lymphoma without Hp are less responsive to Hp eradication, a portion of the

false-negative Hp cases are potentially curable by the Hp eradication therapy alone. Although the rationale for this finding is not fully understood, it is suspected that some Hp-negative MALT lymphomas are false-negatives due to patchy distribution of the micro-organism in the mucosa and due to limited tissue sampling by biopsies (29, 30). In contrast to serology, the UBT may produce false-negative results if performed after the use of Hp- and urease-suppressive therapies, such as PPI and antibiotics (30).

*False-positive results*. Apart from the false-negative results, the UBT also gives false-positive results, which have received more attention during the past 10 years (28, 30-33). These false-positive results may appear in patients with acid-free stomach (due to AG or a long-term PPI use) in particular where urease-positive bacterial species (32, 33) or yeast-like organisms (28) frequently colonize.

This possibility of false-positive UBT results was already known in the late 1990s, when the 2005 Nobel Laureate Barry Marshal described that false-positive UBT results had been reported in patients with gastrectomy, generally related to the presence of urease-positive bacteria other than Hp (11). In the report of Michaud *et al*., UBT was positive and urease-positive bacteria other than Hp were recovered in gastric juice in hypochlorhydric children due to PPI use (34). In general, gastric bacterial overgrowth even with bacterial and fungal bezoars is a constant phenomenon in an acid-free stomach (28). In the late 1990s, it was demonstrated that the number of microbes in gastric mucosa is comparable to that of gastric juice (35), and these non-Hp bacteria are also embedded in the mucus and even in close contact with the gastric epithelium, similarly to Hp (36-38).

In their study, Gurbuz *et al*. compared UBT with histology and the rapid urease test. Histology revealed dense yeast-like micro-organisms in the biopsies from all patients with false-positive UBT results. The authors concluded that gastric mucosal colonization by yeast-like micro-organisms with urease activity can account for the high frequency of false-positive UBT results (31). Brandi *et al*. evaluated the presence of urease-positive bacteria other than Hp in gastric juice and mucosa in 25 hypochlorhydric and 10 control individuals. Convincingly, six hypochlorhydric patients had 10 strains of urease-positive non-Hp bacteria, among which *Staphylococcus capitis urealiticum* showed the strongest urease activity (32).

In another study, the UBT gave false-positive results in 4/102 individuals, shown to be caused by the presence of urease-positive bacteria in the oral cavity and stomach (33). Altogether, five bacterial species with urease activity (*Proteus mirabilis, Citrobacter freundii, Klebsiella pneumoniae, Enterobacter cloacae* and *Staphylococcus aureus*) were isolated from the oral cavity or stomach.

## The SAT

SAT tests are non-invasive diagnostic tests for Hp infection. Two types of SATs exist; one based on enzyme immunoassay and another on immunochromatography (9, 10). SATs do not require expensive chemical agents or specific equipment, which makes them less expensive than UBT. Many guidelines have emphasized that enzyme immunoassay-based SATs using monoclonal antibodies are useful in the primary diagnosis as well as in the assessment of Hp eradication therapy (5, 6, 8). Immunochromatography-based tests do not require particular equipment and are, therefore, also suitable for low-resource settings (5, 6). The accuracy of SATs is lower when the stool samples are unformed or watery, because the Hp-specific antigens in such samples are diluted. The ambient temperature and the interval between sample collection and analysis also affect the SAT results (5-10).

In most settings described above for the UBT, the same potential sources of errors also apply to the SAT (5, 6, 9, 10, 39, 40). The same inherent reasons, *i.e*. a low bacterial load in the stomach, or recent use of PPIs (within 2 weeks) or antimicrobials (within 4 weeks), also contribute to false-negative results in the SAT (14-16). Similarly, bleeding can reduce the sensitivity of the SAT (14, 15). Data from a systematic review suggests repeating of the SAT (after at least 4 weeks) in patients with bleeding ulcer in the case of a negative result (17). In patients with AG, IM, GC, or partial gastrectomy, SAT may have lower accuracy (17).

## The Shortcomings of UBT and SAT Can Be Avoided by Use of a Panel of Serum Biomarkers

It is possible to avoid the shortcomings of the UBT and SAT tests by applying a serological test based on simultaneous assays of multiple stomach-specific serum biomarkers. A Finnish biotechnology company Biohit Oyj (Helsinki, Finland) has developed and extensively validated a test with four gastric biomarkers, namely GastroPanel® (41, 42). In addition to being a serological Hp test, GastroPanel® also measures several distinct biological parameters, including independent biomarkers for i) gastric acid output, ii) intra-gastric acidity, iii) activity of inflammation, and iv) detection of AG. All markers play a key role in stomach physiology, thus reflecting the health or disease of the gastric mucosa. Due to the complex interplay of interdependent biomarkers measured in one and the same serum/plasma sample, the GastroPanel® is free from the typical limitations of UBT and SAT. In addition, this test is capable of diagnosing both AG and Hp infection with their potentially harmful clinical sequelae. Not suffering from the inherent shortcomings of the stand-alone Hp tests, the GastroPanel® is a perfect and reliable non-invasive diagnostic tool for the first-line examination of dyspeptic patients, as well as for the screening of the those at risk for GC (41, 42).

GastroPanel® was introduced in a series of recent communications (37, 38, 43-45). The test is composed of enzyme-linked immunosorbent assays (ELISA) of three biomarkers: pepsinogen I (PGI), pepsinogen II (PGII) and gastrin-17 (G-17), combined with an ELISA assay of Hp (IgG) antibodies, all being measured from the same serum/plasma sample (43-45). It is noteworthy that GastroPanel® includes the assay of G-17, a peptide hormone that is synthesized specifically by antral G-cells and is an excellent biomarker for the structure and function of the antral mucosa. G-17 rapidly reacts to stomach acidity, and its levels in serum/plasma increase in all conditions where the acid content of the stomach is reduced; G-17 will decrease to extremely low levels when the acid output is increased (stomach is hyperacid).

The results of GastroPanel® are interpreted by a special software application (GastroSoft®), another innovation of Biohit Oyj. During the past decade, GastroPanel® has been extensively validated in both diagnostic and screening settings. In a meta-analysis covering all the published literature until 2016 (45), GastroPanel® proved to be a highly accurate test for diagnosis of both AGA and AGC.

## Introduction to the GastroPanel® Examination

GastroPanel® biomarker examination is the best available first-line diagnostic test for Hp infection in general, for examination of patients with dyspepsia, as well as for screening of AG. In other words, GastroPanel® is intended for testing those at increased risk for i) stomach and esophageal cancer; and for ii) malabsorption of vitamin-B12, calcium, iron, magnesium, zinc, and some medicines (due to AG and acid-free stomach) (2, 41, 46-51).

GastroPanel® uses specific monoclonal antibodies to detect stomach-specific biomarkers that regulate the normal stomach physiology. These biomarkers: PGI, PGII, amidated G-17, and Hp antibodies, provide information on both the structure and function of the stomach mucosal compartment (antrum and corpus separately) (46-53). This marker panel also measures the capacity of the corpus and antrum to produce gastric acid and G-17 hormone, thus disclosing the major gastric pathologies and their topography, including the grade and extent of AG and the activity of mucosal inflammation (54-56).

Normal plasma levels of all four biomarkers indicate that the stomach mucosa has a normal structure and function. Abnormal levels are always a sign of a non-healthy stomach, reflecting disturbances in the feedback mechanisms that regulate synthesis and output of gastric acid, 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 useful in distinguishing between the functional disturbances of the antrum (normal or high G-17s) and AGA (G-17s does not react to protein stimulation) (57, 58). For G-17s, the assay in serum/plasma sample is performed 20 minutes after ingestion of a small amount of specific protein.

GastroPanel® is unique in that the results are interpreted by GastroSoft® application (42), capable of linking the biomarker profiles into five possible diagnostic categories reflecting stomach morphology: i) normal mucosa, ii) non-atrophic (Hp) gastritis, iii) AGC, iv) AGA, and v) AG in both the antrum and corpus (49-51, 57-59). GastroPanel® is optimized to cover the same diagnostic categories that are included in the endoscopic and histological classification of chronic gastritis in the Updated Sydney System (USS) in 1996 (60). In addition, GastroSoft® distinguishes three other biomarker profiles that reflect functional disturbances in the acid output of the stomach (see below).

GastroPanel® has been tested in several large trials based on biopsy-confirmed gastroscopies (43, 46, 61) used for validating the cut-off values for each biomarker of the panel. These studies clearly confirm the accuracy of GastroPanel® in detecting the most important endpoint, moderate-to-severe AG (AG2+) (45). Thus, normal values of PGI, PGII and their ratio (PGI/PGII) preclude AGC with a negative predictive value (NPV) of over 95% (43, 46). In turn, the values of PGI, PGII and a PGI/PGII ratio below the established cut-off levels predict AGC2+ with an area under receiver operating characteristics curve exceeding 0.950 in adequately-powered, USS-validated series (43, 45).

## Biomarkers of the GastroPanel®

*Pepsinogen I. PGI* is included in GastroPanel® to identify patients who have AGC, for which the PGI is a highly accurate biomarker (44, 45, 62-65). PGI is a precursor enzyme (zymogen) of pepsin, synthesized by the chief cells and the neck cells of the gastric corpus. As a pepsin precursor, the major part of PGI is excreted into the gastric lumen but a minor fraction is released into the blood circulation. The circulating PGI concentration is closely correlated with the quantity of the chief cells in the corpus, and any loss of these cells due to mucosal atrophy results in a linear decrease in blood PGI levels (62-65). For as yet unknown reasons, AG increases the risk of GC (2, 9, 66-68). In this framework, PGI is also an excellent marker for GC risk. Compared with a healthy stomach, this risk is 5-fold among patients with advanced AGC, but up to 90-fold in those with severe AG in both the antrum and corpus (59, 68, 69).

*Pepsinogen II*. PGII is produced by the chief cells and the mucous neck cells of the gastric corpus, in pyloric glands of the antrum, as well as in Brunner's glands of the proximal duodenum. The PGI/PGII ratio in healthy individuals is between 3 and 20 (51, 56), and decreases linearly with increasing grade of AGC (43, 44, 62, 70), falling below 3.0

when in advanced AGC (AGC2+) (62). The risk of GC is increased (5-fold) when the PGI/PGII ratio is low (41, 52, 55, 65, 71-76). In GastroPanel® test, decreased PGI and low PGI/PGII ratio, along with elevated G-17 is an unfailing hallmark of AGC (37, 38, 44, 45, 50, 51). An elevated PGII level as a stand-alone marker reflects mucosal inflammation, the highest values usually being detected in Hp-associated active gastritis (37, 38). Since the Hp antibody level remains elevated for several months after successful eradication, PGII is also useful in the evaluation of a successful Hp eradication (41, 44, 45).

*Gastrin-17*. Gastrins are linear peptide hormones produced by the G-cells in the duodenum, in the pyloric part of the antrum, and in the pancreas (41, 47). The main function of gastrins is to stimulate the secretion of gastric acid by the parietal cells in the corpus, as well as to increase the motility of the antrum (77). In addition, gastrins are known to stimulate gastric chief cells to secrete PGs and also induce the contraction of the lower esophageal sphincter. Like most peptide hormones, different molecular weight gastrins are synthesized as a result of post-translational modifications from pre-progastrin (47, 77). Thus, a mixture of different molecular weight gastrins is released from the G-cells into the circulation, including G-71, -52, -34, -17, -14, and -6, all of which are carboxy-amidated and circulate in O-sulfated and non-sulfated forms (78). In healthy humans, the dominant forms of gastrin in plasma/serum are amidated G-34 and amidated G-17, the latter, however, being specifically of antral G-cell origin (79). In healthy antrum, G-17 is the most potent form of all gastrins.

The monoclonal antibody included in GastroPanel® detects both amidated, sulfated and non-sulfated forms of G-17, which is a specific biomarker of antral structure and function, and through a negative feedback loop, an indirect biomarker of the corpus as well. Plasma G-17 levels within the normal range indicate normal structure and function of the antrum, whereas low or high values of G-17 reflect abnormalities in acid output by the corpus. The maximum information is obtained when G-17 testing is performed separately for G-17b and G-17s levels, accompanied by PGI, PGII and Hp antibodies of the full GastroPanel® testing (68, 70, 80-84). In Hp-negative individuals, a low G-17b can indicate high acid output. This in turn may increase the risk of peptic ulcer diseases as well as the risk of gastroesophageal reflux disease and Barrett's esophagus, whereas a normal or elevated G-17b excludes the presence of Barrett's esophagus with high probability (84, 85).

*Helicobacter pylori* antibody. Hp infection is the most important cause of chronic gastritis resulting with time in AG. A much more uncommon cause of AG is an autoimmune disease, indicated by low PGI and PGI/PGII ratio, high G-17 and absence of Hp antibodies in the GastroPanel® test (86, 87).

In the stomach, Hp is found within the mucous layer overlying the gastric epithelium, and within the mucosal glands, but it does not appear to invade the epithelial cells. The mucosa underneath and surrounding the areas of Hp colonization is invariably inflamed. This condition is referred to as chronic superficial or non-atrophic gastritis which, if untreated, persists for life (1, 11, 41, 86, 87-95). Without adequate eradication of the bacteria, this chronic inflammatory process leads to AG (5, 6, 9).

## GastroPanel® Results Are Interpreted by GastroSoft®

The GastroPanel® test was developed to correspond with the USS classification of chronic gastritis (60, 96), with five diagnostic categories in both. In addition to these five morphology-associated categories, three other marker profiles can be distinguished by GastroPanel®, specific to functional disturbances in acid output. All eight diagnostic categories are depicted in Table I.

*GastroSoft® software application*. GastroSoft® software application was designed to provide the end users of GastroPanel® with the correct results by integrating the biomarker profiles with the appropriate anamnestic information (42, 97). The user of GastroSoft® may produce the test report simply by completing the GastroPanel® request form (with the relevant clinical information) and filing the biomarker values into the Excel (macro) file. The GastroSoft® report contains the information entered in the form, the biomarker values (and their cut-off levels), as well as a written interpretation of the test results (42, 97).

Correct interpretation of GastroPanel® results by GastroSoft® has two important prerequisites: i) Correct sample processing, storage and delivery to the laboratory, and ii) precise recording of the pertinent anamnestic information on the request form (97). The latter is essential in order to correctly specify the biomarker profiles reflecting functional disturbances and those related to eradication of Hp infection. The clinical information recorded in the request form includes the following specific information: 1) Has an Hp infection been eradicated or not? If yes, was the eradication completed less or more than a year ago? 2) Use of PPI medication: None, occasionally, frequently? If the latter, how many days before GastroPanel sampling did you discontinue using PPI? 3) Do you have symptoms due to high acid output? (no, frequent); and 4) Do you use non-steroidal anti-inflammatory drugs? (no, frequently). With all these data accurately recorded, GastroSoft® correctly integrates the clinical information with the biomarker profile and produces a report classifying the results into one of the eight possible diagnostic categories (Table I).

Table I. *The GastroPanel® biomarker profiles and their diagnostic equivalents. #Values in parentheses indicate the normal reference range of each biomarker.*

| | GastroPanel® biomarker levels# | | | | | | |
|---|---|---|---|---|---|---|---|
| Marker profile | PI (30-160 µg/l)[a] | PII (3-15 µg/l) | PGI/PGII ratio (3-20) | G-17b (1-7 pmol/l) | G-17s (3-30 pmol/l) | Hp IgG antibody titer (<30 EIU) | Interpretation |
| 1 | N | N | N | N | N | N | Healthy mucosa (no atrophy, no Hp infection) |
| 2 | N | N | N | L* | N | N | Healthy mucosa. High acid output. |
| 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 Hp infection, not treated |
| 4b | N | N | N | N | ND | N or H[†] | Hp infection successfully eradicated |
| 4c | N | H | N | H | ND | H | Hp eradication failed |
| 5 | L | L | L | H | ND | N^^ or H | Atrophic gastritis in corpus and fundus (AGC) |
| 6 | N | N | N | L | L | H | Atrophic gastritis in antrum (AGA) |
| 7 | L | L | L | L | L | N^^ or H | Atrophic gastritis in both antrum and corpus (AGpan) |
| 8 | H | H | N | H | ND | N | Short (4- to 10-day) pause in continuous PPI treatment. Rebound in gastric acid output. |

N: Normal; L: low; H: high; Hp: *Helicobacter pylori*; ND: no need for testing; PG: pepsinogen; PPI: proton pump inhibitor. *Test PPI medication for 2 weeks, G-17b should normalize. **Stop medication, G-17b should normalize within 2 weeks. ^Can be elevated due to mucosal inflammation. ^^Can disappear in mucosal atrophy with protracted clinical course. [a]PGI cut-off value of 30 µg/l is consonant with moderate/severe atrophic gastritis (AG). [†]*Helicobacter pylori* antibody levels can remain elevated for months after successful eradication.

## Delivery of Plasma Samples

As to the sample processing, storage and delivery, GastroPanel® test has been adapted to different optional procedures. This flexibility is an advantage that enables the use of this test in different settings varying from full clinical laboratory facilities to private doctors' offices with limited access to laboratory equipment. The possible procedures of the sample processing, storage and delivery are the following.

The use of GastroPanel® stabilizer is the preferred option for preparing EDTA samples for delivery (42, 97). EDTA samples stabilized with GastroPanel® stabilizer enables sample transportation without coolers within 3 days, and with coolers (at 4°C) within 7 days. If GastroPanel® stabilizer is not used, plasma samples must be transported frozen.

## Delivery of Whole Blood Samples

If a facility to centrifuge a blood sample and stabilize the plasma sample is not available at the sampling site, samples may be transported as whole blood following certain precautions (42, 97). If it is not possible to separate the EDTA whole blood sample soon after sample collection and add GastroPanel® stabilizer, sample storage correction provided by the GastroSoft® algorithm can be used. Such a correction will apply to G-17 because it decays relatively quickly when stored and therefore may lead to false interpretation if not corrected for (42, 97).

The transportation of whole blood samples is only advisable where the facilities for processing plasma samples are not available. For the whole blood, two options are possible: i) whole blood samples must be put into an ice box (at 2-8°C) immediately after collection and delivered to the testing laboratory within 24 hours (max.); ii) if whole blood samples cannot be transported in this way, the samples must be transported at ambient temperature to the laboratory within 48 hours (max).

In all cases for delivery of whole blood samples, it is essential that the sampling person records (on the test request form) the precise time and date of blood sample collection, as well as the temperature at which the blood sample was stored/transported. Failure to record this information may result in false interpretation of the G-17 results. When this information is accurately recorded, a correct estimation of the true (at the time of sampling) concentration of G-17 is possible, using the correction algorithm (42).

## Interpretation of the GastroPanel®

*Normal biomarker profile*. With all four biomarkers within the normal reference range, the gastric mucosa functions normally and the mucosal structure is normal. Given that the function of the gastric mucosa is critically dependent on the presence or absence (atrophy) of specific cells responsible for acid output (parietal cells), and for output of pepsinogens (chief cells) or G-17 (G-cells), normal function necessitates

the presence of these cells in normal quantities (37, 38, 41, 44, 47, 55, 58). 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. However, a normal marker profile does not exclude minor (insignificant) gastric abnormalities such as non-specific inflammation, mild irritation, or micro-erosions that do not affect the marker profile (37, 38, 44, 47, 98-100).

*Healthy mucosa, 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 output of G-17 from antral G-cells as a result of a positive feedback loop stimulating acid secretion after a meal (41, 44, 47, 77, 78). Acid output results in progressively lower pH in the stomach contents, and the threshold of pH 2.5 triggers a negative feedback to antral G-cells, signaling them to down-regulate their G-17 secretion (77-79, 81). As a result, G-17 output decreases in parallel with the increasing acid output of the corpus (41, 43, 47, 80). When for any reason the acid content in the corpus remains abnormally high (*e.g.* due to other stimulatory mechanisms), the end result is an abnormally low G-17b secretion from the antral G-cells. Using GastroPanel®, this condition is best diagnosed after a test medication with PPI, when the G-17b should be normalized within approximately 2 weeks of therapy. In a highly acidic milieu with low G-17b, however, the levels of stimulated G-17s will remain within normal limits because the G-cells are intact and capable of increasing G-17 secretion upon protein stimulation (37, 38, 44, 47, 51, 98-100).

*Healthy mucosa, low acid output due to PPI medication*. 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 an elevated blood level of G-17b (44, 47, 80). The two prime conditions leading to low acid output are AGC, and long-term use of PPI medication (or to a lesser extent, H2-receptor blockers). The former is excluded by normal (or even elevated) values of PGI, PGII, and a normal PGI/PGII ratio (37, 38, 44, 47), while the latter is best diagnosed by discontinuing PPI medication. In the latter case, the antral G-17b should be normalized within 2 weeks (44, 47, 77-79, 81).

*Non-atrophic Hp-associated gastritis*. Like bacteria in general, Hp can also induce acute inflammation in the gastric mucosa, usually with onset in the antrum (1, 5, 7, 41, 47, 54, 92-94). With GastroPanel®, three distinct marker profiles are characteristic of Hp infection (Table I).

*Active Hp infection*. In an active Hp infection, the Hp antibody level is raised above the cut-off value (30 EIU), which may be the only abnormal finding, with all other markers falling within the 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 secretory cells, as depicted in Table I (profile 4a) (5, 7, 37, 38, 41, 47, 54, 92-94, 98-100).

*Successful Hp eradication*. Successful Hp eradication by active treatment results in a normalized value for Hp antibodies and the three ("inflammatory") markers (PGI, PGII, G-17) (Table I; 4b), with a delay of some weeks (37, 38, 44, 47, 98-100). In contrast, Hp antibody levels remain elevated for a longer period of time, which is subject to individual variation.

*Failed Hp eradication*. In cases where an Hp eradication attempt fails, the Hp antibody level remains elevated (usually slightly), PGI and the PGI/PGII ratio usually fall within the normal range, whereas PGII or G-17b may remain slightly elevated as a sign of an ongoing inflammatory process (Table I, profile 4c). The result can be confirmed after 5-6 months, followed by a new treatment attempt if indicated (5, 6, 9, 10).

*Atrophic corpus gastritis*. By definition, the loss of chief cells in the oxyntic glands of the corpus, along with the loss of parietal cells 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 glandular cells in the antral mucosa (37, 38, 41, 44, 47). The disproportionate reduction of these two markers will result in a reduced PGI/PGII ratio, which is another excellent signature of AGC (41, 43-45, 47, 52-54, 56, 69). These reduction in PGI and PGI/PGII ratio are progressive and are closely correlated with the severity of AGC, total atrophy and an acid-free stomach being the final endpoint (83, 86). In the case of an intact (normal) antral mucosa, AGC leads to a markedly increased serum level of G-17b (44, 47, 80) (Table I, profile 5). During the protracted course of chronic AGC over decades, Hp itself may disappear from the stomach, resulting in gradual normalization of the Hp antibody level even though the *corpus mucosa* is totally atrophic and the stomach is acid-free (11, 19, 26, 27).

*Atrophic antrum gastritis*. When mucosal atrophy affects the antrum only, all corpus-specific biomarkers will remain within the normal range (Table I). By definition, AGA is caused by Hp infection, and Hp antibodies are invariably elevated in AGA. In AGA, the G-cells are reduced in number and finally disappear, leading to progressively reduced plasma levels of G-17b. In severe AGA, there is no response in G-17 output to protein stimulation (G-17s) because the G-cells are missing (Table I, profile 6) (41, 43, 44, 47, 61, 63, 80, 83, 85, 98-101). Thus, the distinction between the two

potential causes of low G-17b: i) high acid output (profile 2) and ii) AGA (profile 6), is neatly made after ordinary GastroPanel® by using G-17s testing with protein stimulation (37, 38, 42, 44, 47, 98-100). As pointed out, G-17s will react normally (increase) only in the former, but fails to increase in severe AGA.

*Atrophic gastritis of the antrum and corpus*. The most severe form of AG is that affecting both the antrum and corpus (37, 38, 41, 44, 47, 60, 96). As an end result, the chief cells in the corpus and G-cells in the antrum disappear, leading to a biomarker expression profile where both PGI, PGII and G-17 are substantially reduced (Table I, profile 7) (37, 38, 41, 44, 47, 84-88, 98-100). This applies to both G-17b and G-17s, which remain low even after protein stimulation. Like in AGC, the Hp antibody level can remain within a normal range or elevated depending on the severity of atrophy in the antrum and corpus.

*Panel profiles caused by PPI medication*. Any gastric acid-suppressive medication will inevitably interfere with the profile of the GastroPanel® markers. To enable an unbiased assessment of the biomarker profiles, the manufacturer recommends that the patient discontinues any acid-suppressive treatment for 10 days before serum/plasma sampling (37, 38, 44, 47, 97-100). This withdrawal of PPI or H2-blocker medication is not always possible because of intractable symptoms, and to acknowledge this, the latest version of GastroSoft® was designed to take into account the continued use of PPI medication. In this context, important pieces of knowledge are i) an accurate recording of the PPI/$H_2$-medication, ii) information of whether or not the medication was discontinued, and if so, iii) for how many days before serum/plasma sampling. With this information accurately recorded, GastroSoft® is capable of interpreting the test results correctly, defined as profile 8 in Table I (42).

PPI and $H_2$-blockers effectively reduce gastric acid production by parietal cells (37, 38, 47, 51). This increases the production of G-17b and also the output of PGs. Once the PPI/$H_2$-blocker treatment is discontinued, it takes 4-10 days for acid output and G-17b levels to normalize. However, PGs respond more slowly, and PGI and PGII levels may remain above the cut-off values for up to 2-3 weeks (37, 38, 44, 47, 98-100). Of note, an abrupt termination of long-term PPI medication is typically followed by rebound acid hypersecretion, frequently accompanied by symptoms of hyper-acidity and extremely low levels of G-17b (41, 44, 45, 47, 80).

## Clinical Performance Confirmed in Two Recent Meta-Analyses

To provide an estimate of the diagnostic accuracy of the GastroPanel® test, we recently performed a systematic review and meta-analysis of all published studies accumulated since the early 2000s (45). Studies were eligible, if i) GastroPanel® test (instead of stand-alone markers) was used to diagnose biopsy-confirmed AGC or AGA, and ii) exact numbers of patients were available to enable calculating the sensitivity and specificity. Altogether, 27 studies were eligible, comprising 8.654 tested patients from different geographic regions. Significant heterogeneity between studies reporting AGC (n=27) or AGA (n=13) warranted random-effects model for the summary statistics. GastroPanel® was shown to perform better in diagnosis of AGC than AGA, with 70.2% *vs*. 51.6% pooled sensitivity, and 93.9% *vs*. 84.1% pooled specificity, respectively (45). In analysis for publication bias, it was determined there were a few hypothetically missing studies, but these had only a marginal effect on the pooled sensitivity and specificity estimates. The results of this first meta-analysis clearly corroborate the consensus statement of the international experts published in 2012 (41).

The second meta-analysis was published by a group of gastroenterologists in Italy (102). Similarly to the first meta-analysis (45), these authors also assessed the performance of the GastroPanel® test in diagnosis of AG. Studies were eligible if they assessed the accuracy of the GastroPanel® test for the diagnosis of AG using the endoscopic and histological USS in classification of chronic gastritis as reference (60, 96). The authors identified 20 eligible studies with a total of 4,241 subjects tested with the biomarker panel for AG, regardless of its topographic location (AGA or AGC) in the stomach (102). The pooled sensitivity was 74.7% (95% CI=62.0-84.3%) and the pooled specificity was 95.6% (95% CI=92.6-97.4%). The Authors concluded that the GastroPanel® test is a reliable tool for the diagnosis of AG, and applicable for both screening of those individuals or populations at high-risk for GC (102).

## GastroPanel® Examination Is Devoid of the Limitations of Conventional Hp Tests

Diagnosis and therapy of Hp has been exhaustively reviewed (5, 6, 9, 15-19, 26, 27, 88-81, 103). The message is unanimous in that several clinical conditions can seriously hamper the diagnostic value of the UBT and SAT (98-100). Basically, these false-negative results are due to decreased bacterial loads in the stomach mucosa and include the following clinical conditions: i) PPI medication; ii) use of antibiotics; iii) bleeding peptic ulcer; iv) AG and hypoacid stomach; v) GC; vi) MALT lymphoma, and vii partial gastrectomy. Under these circumstances, neither UBT nor SAT (or Hp serology) detects active Hp infection and both are incapable of diagnosing AG. These two tests miss or overlook the patients at highest risk for the potentially serious clinical sequelae of Hp infection and AG, namely: i)

GC and its precursor lesions, ii) esophageal cancer, iii) vitamin-B12 deficiency, and iv) malabsorption of calcium, iron, magnesium and certain medicines (98-100). Importantly, even successful eradication of Hp infection does not interfere with the clinical course of AG, leaving patients with missed AG at constant high risk for GC (6, 48).

GastroPanel® is devoid of the diagnostic drawbacks of the UBT and SAT (20-40, 98-100). GastroPanel® is the most comprehensive Hp test, with the Hp antibody assay being complemented by three stomach-specific biomarkers. Depending on the stage of infection, three different marker profiles can be seen in association with Hp infection (Table I). In an active Hp infection, the Hp antibody level is raised, which may be the only abnormal finding in GastroPanel® (37, 38, 42-44). Frequently, however, an active ongoing Hp infection causes an inflammatory reaction severe enough to increase the levels of the inflammatory markers PGI, PGII and G-17 (42-44). Secondly, a successful Hp eradication by active treatment should result in normalized values of all three markers, with a delay of some weeks to months. This delay should be taken into account while interpreting the GastroPanel® results in samples taken soon after Hp eradication (42-44, 98-100). Finally, in cases where Hp eradication fails, the Hp antibody level remains elevated, accompanied by slightly elevated PGII or G-17b values due to a persistent inflammatory reaction.

## Conclusion

GastroPanel® test has been on the market for over 10 years, and the test is the first non-invasive diagnostic tool based on physiology of three biomarkers specific to stomach structure and function, complemented by ELISA (IgG) testing for Hp antibodies (5, 6, 9, 10, 104). The test panel exploits the understanding of the natural history of gastritis provided by long-term cohort studies (19, 26, 27, 59, 62, 63, 68, 70, 80, 82, 85-87, 91, 96).

With all these sophisticated diagnostic properties, this panel of four biomarkers makes the GastroPanel® examination also the most comprehensive Hp test, devoid of the known shortcomings of the conventional Hp tests (20-40, 98-100). The International *Helicobacter pylori* Study Group has stated in their reports on Maastricht IV and V Consensus Conferences (2012 and 2016), that blood biomarker tests are a reliable means to identify and screen for gastric diseases and their risk status (6, 104). In the recently published US consensus report on Hp diagnosis, GastroPanel® test was addressed only briefly, most likely because it has not yet been submitted for FDA-approval in the USA (105).

In 2012, 16 experts from 12 countries in the Healthy Stomach Initiative (http:\www.hsinitiative.org) published a position paper with a set of recommendations implicating that this biomarker test is suitable for both screening of asymptomatic patients and for diagnosis of dyspeptic patients (41). In addition to these international consensus statements, expert reports along similar lines have been published by several prominent authorities (106, 107). Accordingly, in 2006, Pasechnikov *et al*. made the following pertinent conclusions: "The analysis of the literature data and results of our own research allow us to conclude that the serious medical and ethical problems of the "test and treat" strategy can be corrected simply and economically by replacing its $^{13}$C-urea breath test or stool antigen test by the GastroPanel examination" (106). Similarly, Talley *et al*. indicated that "in many countries, such as Sweden and the US, the "test and treat" strategy alone is not considered sufficient. The Hp tests of the "test and treat" strategy do not find AG and related risks, such as GC and precancerous lesions, which should be confirmed by gastroscopy and biopsy specimen examination. Consequently, GastroPanel and gastroscopy with biopsies reveal patients with precancerous lesions and early-stage GC, and, therefore, save people from unnecessary deaths because of gastric cancer" (107).

In addition to the fact that GastroPanel® testing increases patient safety, systematic use of this test will also enable substantial savings in healthcare costs (111). According to the cost-efficiency model developed by the Nordic Healthcare Group, organized GastroPanel® screening for the GC risks of *e.g*. one age group sized 67,837 (50-year-old) people would save over 80 million euros in life-time healthcare costs in Finland (https://www.gastropanel.com/decision-makers/screening-model).

Given that the Hp infection is the single most important risk factor of GC, it is plausible to consider GastroPanel® examination as the most comprehensive diagnostic test of Hp infection since the test is i) free from the shortcomings of conventional Hp tests, and ii) provides added diagnostic value by also detecting AG and acid-free stomach (45, 102, 108-110), *i.e*. not missing patients who are at the highest risk for GC and who escape medical attention when UBT and SAT are used.

## References

1 Marshall BJ and Warren JR: Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet *1(8390)*: 1311-1315, 1984. PMID: 6145023

2 Correa P, Haenszel W, Cuello C, Zavala D, Fontham E and Zarama G: Gastric precancerous process in a high risk population: cohort follow-up. Cancer Res *50(15)*: 4737-4740, 1990. PMID: 2369748

3 Hayakawa Y, Sethi N, Sepulveda AR, Bass AJ and Wang TC: Oesophageal adenocarcinoma and gastric cancer: Should we mind the gap? Nat Rev Cancer *16(5)*: 305-318, 2016. PMID: 27112208, DOI: 10.1038/nrc.2016.24

4 Ohata H, Kitauchi S and Yoshimura N: Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. Int J Cancer *109(1)*: 138-143, 2004. PMID: 14735480, DOI: 10.1002/ijc.11680

5 Malfertheiner P, Megraud F and O'Morain C: Current European concepts in the management of *Helicobacter pylori* infection-The Maastricht consensus report. The European *Helicobacter pylori* Study Group (EHPSG). Eur J Gastroenterol Hepatol *9(1)*: 1-2, 1997. PMID: 9031888

6 Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R, Moayyedi P. Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K and El-Omar EM: on behalf of the European *Helicobacter* and Microbiota Study Group and Consensus panel. Management of *Helicobacter pylori* infection - the Maastricht V/Florence Consensus Report. Gut *66(1)*: 6-30, 2017. PMID: 27707777, DOI: 10.1136/gutjnl-2016-312288

7 Fritz N, Birkner B and Schusdziarra V: Are guidelines followed in *Helicobacter pylori* diagnosis and therapy? An inquiry among gastroenterologists, referring physicians and patients in Munich. Z fur Gastroenterol *38(5)*: 349-355, 2000. PMID: 10875143, DOI: 10.1055/s-2000-14876

8 Zagari RM, Romano M, Ojetti V, Stockbrugger R, Gullini S, Annibale B, Farinati F, Ierardi E, Maconi G, Rugge M, Calabrese C, Di Mario F, Luzza F, Pretolani S, Savio A, Gasbarrini G and Caselli M: Guidelines for the management of *Helicobacter pylori* infection in Italy: The III Working Group Consensus Report 2015. Dig Liver Dis *47(11)*: 903-912, 2015. PMID: 26253555, DOI: 10.1016/j.dld.2015.06.010

9 Malfertheiner P, Megraud F and O'Morain C: Current concepts in the management of *Helicobacter pylori* infections. The Maastricht 2-2000 Consensus Report. Aliment Pharmacol Ther *16(2)*: 167-180, 2002. PMID: 11860399

10 Malfertheiner P, Megraud F and O'Morain C: Current concepts in the management of *Helicobacter pylori* infection: the Maastricht III Consensus Report. Gut *56(6)*: 772-781, 2007. PMID: 17170018, DOI: 10.1136/gut.2006.101634

11 Marshall B. The 14C urea breath test. *In*: Lee A, Megraud F. (eds). *Helicobacter pylori*: Techniques for Clinical Diagnosis and Basic Research. 2nd Ed. London: WB Saunders Company, pp. 83-93, 1996.

12 Gisbert JP and Pajares JM: 13C-Urea breath test in the management of *Helicobacter pylori* infection. Dig Liver Dis *37(12)*: 899-906, 2005. PMID: 16280266, DOI: 10.1016/j.dld.2005.09.006

13 Ferwana M, Abdulmajeed I and Alhajiahmed A: Accuracy of urea breath test in *Helicobacter pylori* infection: Meta-analysis. World J Gastroenterol *21(4)*: 1305-1314, 2015. PMID: 25632206, DOI: 10.3748/wjg.v21.i4.1305

14 Levine A, Shevah O and Shabat-Sehayek V: Masking of 13C-urea breath test by proton pump inhibitors is dependent on type of medication: comparison between omeprazole, pantoprazole, lansoprazole and esomeprazole. Aliment Pharmacol Therapeut *20(1)*: 117-122, 2004. PMID: 15225178, DOI: 10.1111/j.1365-2036.2004.02021.x

15 Asfeldt AM, Lochen ML and Straume B: Accuracy of monoclonal antibody-based stool antigen test in the diagnosis of *Helicobacter pylori* infection. Scand J Gastroenterol *39(11)*: 1073-1077, 2004. PMID: 15545164, DOI: 10.1080/00365520410007944

16 Vaira D, Gatta L and Ricci C: *Helicobacter pylori*: Diseases, tests and treatment. Digest Liver Dis *33(9)*: 788-794, 2001. PMID: 11838615

17 Sanchez-Delgado J, Genè E and Suarez D: Has HP prevalence in bleeding peptic ulcer been underestimated? A meta-regression. Am J Gastroenterol *106(3)*: 398-405, 2011. PMID: 21304499, DOI: 10.1038/ajg.2011.2

18 Lehours P, Ruskone-Fourmestraux A and Lavergne A: Which test to use to detect *Helicobacter pylori* infection in patients with low-grade gastric mucosa-associated lymphoid tissue lymphoma? Am J Gastroenterol *98(2)*: 291-295, 2003. PMID: 12591043, DOI: 10.1111/j.1572-0241.2003.t01-1-07264.x

19 Kokkola A, Rautelin H, Puolakkainen P, Sipponen P, Färkkilä M, Haaplainen R and Kosunen TU: Positive result by serology indicates active *Helicobacter pylori* infection in patients with atrophic gastritis. J Clin Microbiol *36(6)*: 1808-1810, 1998. PMID: 9620430

20 Ozturk E, Yesilova Z and Ilgan S: Performance of acidified 14C-urea capsule breath test during pantoprazole and ranitidine treatment. J Gastroenterol Hepatol *24(7)*: 1248-1251, 2009. PMID: 19486449 DOI: 10.1111/j.1440-1746.2009.05845.x

21 Graham DY, Opekun AR and Jogi M: False negative urea breath tests with H2-receptor antagonists: interactions between *Helicobacter pylori* density and pH. Helicobacter *9(1)*: 17-27, 2004. PMID: 15156900

22 Perri F, Maes B, Geypens B, Ghoos Y, Hiele M and Rutgeerts P: The influence of isolated doses of drugs, feeding and colonic bacterial ureolysis on urea breath test results. Aliment Pharmacol Ther *9(6)*: 705-709, 1995. PMID: 8824660

23 Leung WK, Hung LC, Kwok CK, Leong RW, Ng DK and Sung JJ: Follow-up of serial urea breath test results in patients after consumption of antibiotics for non-gastric infections. World J Gastroenterol *8(4)*: 703-706, 2002. PMID: 12174382

24 Gisbert JP, Esteban C, Jimenez I and Moreno-Otero R: $^{13}$C-Urea breath test during hospitalization for the diagnosis of *Helicobacter pylori* infection in peptic ulcer bleeding. *Helicobacter 12(3)*: 231-237, 2007. PMID: 17493003, DOI: 10.1111/j.1523-5378.2007.00492.x

25 Barkun AN, Bardou M and Kuipers EJ: International consensus recommendations on the management of patients with nonvariceal upper gastrointestinal bleeding. Ann Intern Med *152(2)*: 101-113, 2010. PMID: 20083829, DOI: 10.7326/0003-4819-152-2-201001190-00009

26 Kokkola A, Rautelin H, Puolakkainen P, Sipponen P, Färkkilä M, Haapiainen R and Kosunen TU: Diagnosis of *Helicobacter pylori* infection in patients with atrophic gastritis: comparison of histology, $^{13}$C-urea breath test, and serology. Scand J Gastroenterol *35(2)*: 138-141, 2000. PMID: 10720110

27 Kokkola A, Kosunen TU, Puolakkainen P, Sipponen P, Harkonen M, Laxen F, Virtamo J, Haapiainen R and Rautelin H: Spontaneous disappearance of *Helicobacter pylori* antibodies in patients with advanced atrophic corpus gastritis. APMIS *111(6)*: 619-624, 2003. PMID: 12969017

28 Lahner E, Vaira D, Figura N, Pilozzi E, Pasquali A, Severi C, Perna F, Delle Fave G and Annibale B: Role of noninvasive tests (13C-Urea Breath Test and Stool Antigen Test) as additional tools in diagnosis of *Helicobacter pylori* infection in patients with atrophic body gastritis. *Helicobacter 9(5)*: 436-442, 2004. PMID: 15361083, DOI: 10.1111/j.1083-4389.2004.00262.x

29 Franco M, Rugge M, D'Andrea E, Mescoli C, Menin C and Farinati F: Gastric mucosa-associated lymphoid tissue lymphoma and *Helicobacter pylori*: Scratch and win. Scand J Gastroenterol *40(1)*: 115-119, 2005. PMID: 15841725

30 Garza-González E, Perez-Perez GI, Maldonado-Garza HJ and Bosques-Padilla FJ: A review of *Helicobacter pylori* diagnosis, treatment, and methods to detect eradication. World J Gastroenterol *20(6)*: 1438-1449, 2014. PMID: 24587620, DOI: 10.3748/wjg.v20.i6.1438

31 Gurbuz AK, Ozel AM, Narin Y, Yazgan Y, Baloglu H and Demirturk L: Is the remarkable contradiction between histology and $^{14}C$ urea breath test in the detection of *Helicobacter pylori* due to false-negative histology or false-positive $^{14}C$ urea breath test? J Int Med Res *33(6)*: 632-640, 2005. PMID: 16372580 DOI: 10.1177/147323000503300604

32 Brandi G, Biavati B, Calabrese C, Granata M, Nannetti A, Mattarelli P, Di Febo G, Saccoccio G and Biasco G: Urease-positive bacteria other than *Helicobacter pylori* in human gastric juice and mucosa. Am J Gastroenterol *101(8)*: 1756-1761, 2006. PMID: 16780553, DOI: 10.1111/j.1572-0241.2006.00698.x

33 Osaki T, Mabe K, Hanawa T and Kamiya S: Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of *Helicobacter pylori* infection. J Med Microbiol *57(Pt 7)*: 814-819, 2008. PMID: 18566138 DOI: 10.1099/jmm.0.47768-0

34 Michaud L, Gottrand F and Ganga-Zandzou PS: Gastric bacterial overgrowth is a cause of false-positive diagnosis of *Helicobacter pylori* infection using 13C urea breath test. Gut *42(4)*: 594-595, 1998. PMID: 9616328

35 Brandi G, Biasco G and Biavati B: Bacterial colonization in juice and biopsies of the achlorhydric stomach. Gastroenterol *108(suppl 1)*: A787, 1995.

36 Brandi G, Pisi and Biasco G: Bacteria in biopsies in humans hypochlorhydric stomach: A scanning electron microscopy study. Ultrastruct Pathol *20(3)*: 203-209, 1996. PMID: 8727062

37 Syrjänen K: Serological biomarker panel (GastroPanel®): A test for non-invasive diagnosis of dyspeptic symptoms and for comprehensive detection of *Helicobacter pylori* infection. Biomark J *3*: 1-10, 2017. doi: 10.21767/2472-1646.100024

38 Syrjänen K: Role of serological biomarker testing (GastroPanel®) in diagnosis of symptomatic dyspepsia and in screening of the risks of stomach cancer. EC Gastroenterol Digest Sys*t 1(6)*: 209-222, 2017. https://www.ecronicon.com/ecgds/pdf/ECGDS-01-000034.pdf

39 Erzin Y, Altun S and Dobrucali A: Evaluation of two enzyme immunoassays for detecting *Helicobacter pylori* in stool specimens of dyspeptic patients after eradication therapy. J Med Microbiol *54(Pt 9)*: 863-866, 2005. PMID: 16091438 DOI: 10.1099/jmm.0.45914-0

40 Graham DY, Opekun AR, Hammoud F. Studies regarding the mechanism of false-negative urea breath tests with proton pump inhibitors. Am J Gastroenterol *98(5)*: 1005-1009, 2003. PMID: 12809820, DOI: 10.1111/j.1572-0241.2003.07426.x

41 Agréus L, Kuipers EJ, Kupcinskas L, Malfertheiner P, Di Mario F, Leja M, Mahachai V, Yaron N, van Oijen M, Perez Perez G, Rugge M, Ronkainen J, Salaspuro M, Sipponen P, Sugano K and Sung J: Rationale in diagnosis and screening of atrophic gastritis with stomach-specific plasma biomarkers. Scand J Gastroenterol *47(12)*: 136-147, 2012. PMID: 22242613, DOI: 10.3109/00365521.2011.645501

42 http://www.gastropanel.com

43 Väänänen H, Vauhkonen M, Helske T, Kääriäinen I, Rasmussen M, Tunturi-Hihnala H, Koskenpato J, Sotka M, Turunen M, Sandström R, Ristikankare M, Jussila A and Sipponen P: Non-endoscopic diagnosis of atrophic gastritis with a blood test. Correlation between gastric histology and serum levels of gastrin-17 and pepsinogen I: a multi-centre study. Eur J Gastroenterol Hepatol *15(8)*: 885-891, 2003. PMID: 12867799, DOI: 10.1097/01.meg.0000059169.46867.01

44 Syrjänen KJ, Sipponen P, Härkönen M, Peetsalu A and Korpela S: Accuracy of GastroPanel® testing in detection of atrophic gastritis. Eur J Gastroenterol Hepatol *27(1)*: 102-104, 2015. PMID: 25426982, DOI: 10.1097/MEG.0000000000000215

45 Syrjänen K: A Panel of serum biomarkers (GastroPanel®) in non-invasive diagnosis of atrophic gastritis. Systematic review and meta-analysis. Anticancer Res *36(10)*: 5133-5144, 2016. PMID: 27798873, DOI: 10.21873/anticanres.11083

46 Storskrubb T, Aro P, Ronkainen J, Sipponen P, Nyhlin H and Talley NJ: Serum biomarkers provide an accurate method for diagnosis of atrophic gastritis in a general population: The Kalixanda study. Scand J Gastroenterol *43(12)*: 448-1455, 2008. PMID: 18663663, DOI: 10.1080/00365520802273025

47 Wikström M: Assessment of stomach health by "chemical gastroscopy". Eur Gastroenterol Rev *2*: 1-6, 2012.

48 Sipponen P, Härkönen M and Salaspuro M: Atrofinen gastriitti jää usein liian vähälle huomiolle. Suomen Lääkärilehti *63(15)*: 1428-1430, 2008. ISSN 0039-5560

49 Suovaniemi O: GastroPanel®-tutkimus osaksi dyspepsian hoitokäytäntöä. Yleislääkäri *4*: 104-106, 2007.

50 Suovaniemi O: State of the art GastroPanel® and Acetium® innovations for the unmet need. Terveyspalvelut *3-4*: 1-12, 2011.

51 Lomba-Viana R, Dinis-Ribeiro M, Fonseca F, Vieira AS, Bento MJ and Lomba-Viana H: Serum pepsinogen test for early detection of gastric cancer in a European country. Eur J Gastroenterol Hepatol *24(1)*: 37-41, 2012. PMID: 21989121, DOI: 10.1097/MEG.0b013e32834d0a0a

52 Miki K: Gastric cancer screening using the serum pepsinogen test method. Gastric Cancer *9(4)*: 245-253, 2006. PMID: 17235625, DOI: 10.1007/s10120-006-0397-0

53 Bornschein J, Selgrad M, Wex T, Kuester D and Malfertheiner P: Serological assessment of gastric mucosal atrophy in gastric cancer. BMC Gastroenterol *12*: 10, 2012. PMID: 22289789, doi: 10.1186/1471-230X-12-10, 2012.

54 Germaná B, Di Mario F, Cavallaro LG, Moussa AM, Lecis P, Liatoupolou S, Comparato G, Carloni C, Bertiato G, Battiestel M, Papa N, Aragona G, Cavestro GM, Iori V, Merli R, Bertolini S, Caruana P and Franzé A: Clinical usefulness of serum pepsinogens I and II, gastrin-17 and anti-*Helicobacter pylori* antibodies in the management of dyspeptic patients in primary care. Dig Liver Dis *37(7)*: 501-508, 2005. PMID: 15975537, DOI: 10.1016/j.dld.2005.01.016

55 Miki K, Ichinose M, Shimizu A, Huang SC, Oka H, Furihata C, Matsushima T and Takahashi K: Serum pepsinogens as a screening test of extensive chronic gastritis. Gastroenterol Jpn *22(2)*: 133-141, 1987. PMID: 3596151

56 Samloff IM, Varis K, Ihamäki T, Siurala M and Rotter JI: Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. Gastroenterol *83(1 Pt)*: 204-209, 1982. PMID: 7084603

57 Korstanje A, den Hartog G, Biemond I and Lamers CB: The serological gastric biopsy: A non-endoscopical diagnostic approach in management of the dyspeptic patient: Significance for primary care based on a survey of the literature. Scand J Gastroenterol *236(Suppl)*: 22-26, 2002. PMID: 12408500

58 Oksanen A, Sipponen P, Miettinen A, Sarna S and Rautelin H: Evaluation of blood tests to normal gastric mucosa. Scand J Gastroenterol *35(8)*: 791-795, 2000. PMID: 10994615

59 Varis K, Sipponen P, Laxén F, Samloff IM, Huttunen JK, Taylor PR, Heinonen OP, Albanes D, Sande N, Virtamo J and Härkönen M and The Helsinki Gastritis Study Group: Implications of serum pepsinogen I in early endoscopic diagnosis of gastric cancer and dysplasia. Scand J Gastroenterol *35(9)*: 950-956, 2000. PMID: 11063155

60 Dixon MF, Genta RM, Yardley JH and Correa P: Classification and Grading of Gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol *20(10)*: 1161-1181, 1996. PMID: 8827022

61 Telaranta-Keerie A, Kara R, Paloheimo L, Härkönen M and Sipponen P: Prevalence of undiagnosed advanced atrophic corpus gastritis in Finland: An observational study among 4,256 volunteers without specific complaints. Scand J Gastroenterol *45(9)*: 1036-1041, 2010. PMID: 20446846, DOI: 10.3109/00365521.2010.487918

62 Varis K, Samloff IM, Ihamäki T and Siurala M: An appraisal of tests for severe atrophic gastritis in relatives of patients with pernicious anemia. Dig Dis Sci *24(3)*: 187-191, 1979. PMID: 456206

63 Varis K, Kekki M, Härkönen M, Sipponen P and Samloff IM: Serum pepsinogen I and serum gastrin in screening of atrophic pangastritis with high risk of gastric cancer. Scand J Gastroenterol *186(1)*: 117-123, 1991. PMID: 1759119

64 Knight T, Wyatt J, Wilson A, Greaves S. Newell D, Hengels K, Corlett M, Webb B, Forman D and Elder J: *Helicobacter pylori* gastritis and serum pepsinogen levels in a healthy population: Development of a biomarker strategy for gastric atrophy in high groups. Br J Cancer *73(6)*: 819-824, 1996. PMID: 8611388

65 Borch K, Axelsson CK, Halgreen H, Damkjaer Nielsen MD, Ledin T and Szesci PB: The ratio of pepsinogen A to pepsinogen C: A sensitive test for atrophic gastritis. Scand J Gastroenterol *24(7)*: 870-876, 1989. PMID: 2799289

66 Correa P: Human gastric carcinogenesis: A multistep and multifactorial process. First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res *52(24)*: 6735-6740, 1992. PMID: 1458460

67 Wong BC, Lam SK and Wong WM: China Gastric Cancer Study Group. *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. JAMA *291(2)*: 187-194, 2004. PMID: 14722144, DOI: 10.1001/jama.291.2.187

68 Sipponen P, Kekki M, Haapakoski J, Ihamäki T and Siurala M: Gastric cancer risk in chronic atrophic gastritis: Statistical calculations of cross-sectional data. Int J Cancer *35(2)*: 173-177, 1985. PMID: 3871738

69 Dinis-Ribeiro M, Yamaki G, Miki K, Costa-Pereira A, Matsukawa M and Kurihara M: Meta-analysis on the validity of pepsinogen test for gastric carcinoma, dysplasia or chronic atrophic gastritis screening. J Med Screen *11(3)*: 141-147, 2004. PMID: 15333273 DOI: 10.1258/0969141041732184

70 Varis K: Surveillance of pernicious anemia. *In*: Precancerous Lesions of the Gastrointestinal Tract. Scherlock P, Morson PC, Barbara L and Veronesi U (eds.): Raven Press, New York, pp. 189-194, 1983.

71 Hattori Y, Tashiro H, Kawamoto T and Kodama Y: Sensitivity and specificity of mass screening for gastric cancer using the measurement of serum pepsinogens. Jpn J Cancer Res *86(12)*: 1210-1215, 1995. PMID: 8636012

72 Yoshihara M, Sumii K, Haruma K, Kiyohira K, Hattori N, Tanka S, Kajiyama G and Shigenobu T: The usefulness of gastric mass screening using serum pepsinogen levels compared with photofluorography. Hiroshima J Med Sci *46(2)*: 81-86, 1997. PMID: 9232936

73 Kodoi A, Yoshihara M, Sumii K, Haruma K and Kajiyama G: Serum pepsinogen in screening for gastric cancer. J Gastroenterol *30(4)*: 452-460, 1995. PMID: 7550854

74 Aoki K, Misumi J, Kimura T, Zhao W and Xie T: Evaluation of cut-off levels for screening of gastric cancer using serum pepsinogens and distribution of levels of serum pepsinogen I, II and of PG1/PGII ratios in a gastric cancer case–control study. J Epidemiol *7(3)*: 143-151, 1997. PMID: 9337512

75 Kikuchi S, Wada O, Miki K, Nakajima T, Nishi T, Kobayashi O and Inaba Y: Serum pepsinogen as a new marker for gastric carcinoma among young adults. Research Group on Prevention of Gastric Carcinoma Among Young Adults. Cancer *73(11)*: 2695-2702, 1994. PMID: 8194008

76 Farinati F, Di Mario F, Plebani M, Cielo R, Fanton MC, Valiante F, Masiero M, DeBoni M, Della Libera G and Burlin A: Pepsinogen A/pepsinogen C or pepsinogen A multiplied by gastrin in the diagnosis of gastric cancer. Ital J Gastroenterol *23*: 194-206, 1991.

77 Rozengurt E and Walsh JH: Gastrin, CCK, signaling, and cancer. Annu Rev Physiol 63: 49-76, 2001. PMID: 11181948, DOI: 10.1146/annurev.physiol.63.1.49

78 Sawada M and Dickinson CJ: The G cell. Annu Rev Physiol *59*: 273-298, 1997. PMID: 9074765, DOI: 10.1146/annurev.physiol.59.1.273

79 Goetze, JP, Paloheimo LI, Linnala A, Sipponen P, Hansen CP and Rehfeld JF: Gastrin-17 specific antibodies are too specific for gastrinoma diagnosis but stain G-cells. Scand J Gastroenterol *40(5)*: 596-598, 2005. PMID: 16036513, DOI: 10.1080/00365520510015449

80 Sipponen P, Ranta P, Helske T, Kääriäinen I, Mäki T and Linnala A: Serum levels of amidated gastrin-17 and pepsinogen I in atrophic gastritis: an observational case-control study. Scand J Gastroenterol *37(7)*: 785-791, 2002. PMID: 12190091

81 Hallissey MT, Dunn JA and Fielding JW: Evaluation of pepsinogen A and gastrin-17 as markers of gastric cancer and high-risk pathologic conditions. Scand J Gastroenterol *29(12)*: 1129-1134, 1994. PMID: 7886402

82 Sipponen P: Gastric cancer: Pathogenesis, risks, and prevention. J Gastroenterol *37(Suppl 13)*: 39-44, 2002. PMID: 12109664

83 Sipponen P, Härkönen M, Alanko A and Suovaniemi O: Diagnosis of atrophic gastritis from a serum sample. Clin Lab *48(9-10)*: 505-515, 2002. PMID: 12389711

84 Sipponen P, Vauhkonen M, Helske T, Kääriäinen I and Härkönen M: Serum fasting level of gastrin-17 is low with long-segment Barrett's esophagus. Gastroenterol *126(4)(Suppl. 2)*: A-177 (S1234), 2004.

85 Sipponen P, Valle J, Varis K, Kekki M, Ihamäki T and Siurala M: Fasting levels of serum gastrin in different functional and morphological states of the antro-fundal mucosa. An analysis of 860 subjects. Scand J Gastroenterol *25(5)*: 513-519, 1990. PMID: 2359980

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

87 Sipponen P: *Helicobacter pylori* gastritis-epidemiology. J Gastroenterol *32*: 273-277, 1997. PMID: 9085182

88 Sipponen P and Marshall BJ: Gastritis and gastric cancer. Western countries. Gastroenterol Clin North Am *29(3)*: 579-592, 2000. PMID: 11030074

89 Wadström T: An update on *Helicobacter pylori*. Curr Opin Gastroenterol *11*: 69-75, 1995. PMID: 7861863

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

91 Sipponen P: Update on the pathologic approach to the diagnosis of gastritis, gastric atrophy, and *Helicobacter pylori* and its sequelae. J Clin Gastroenterol *32(3)*: 96-202, 2001. PMID: 11246343

92 Malfertheiner P, Sipponen P and Naumann M: H. pylori-Gastric Cancer Task Force. *Helicobacter pylori* eradication has the potential to prevent gastric cancer: a state-of-the-art critique. Am J Gastroenterol *100(9)*: 2100-2115, 2005. PMID: 16128957, DOI: 10.1111/j.1572-0241.2005.41688.x

93 Sande N, Nikulin M, Nilson I, Wadström T, Laxen F, Härkönen M, Suovaniemi O and Sipponen P: Increased risk of developing atrophic gastritis in patients infected with CagA+ *Helicobacter pylori*. Scand J Gastroenterol *36(9)*: 928-933, 2001. PMID: 11521982

94 Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N and Sibley RK: *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med *325(16)*: 1127-1131, 1991. PMID: 1891020, DOI: 10.1056/NEJM199110173251603

95 Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH and Friedman GD: *Helicobacter pylori* infection and gastric lymphoma. N Engl J Med *330(18)*: 1267-1271, 1994. PMID: 8145781, DOI: 10.1056/NEJM199405053301803

96 Sipponen P and Price AB: The Sydney system for classification of gastritis 20 years ago. J Gastroenterol Hepatol *26(Suppl 1)*: 31-34, 2011. PMID: 21199511, DOI: 10.1111/j.1440-1746. 2010.06536.x

97 https://www.gastropanel.com/healthcare-professionals-and-laboratories/ gastropanel-report-gastrosoft

98 Syrjänen K and Eronen K: Serological testing in management of dyspeptic patients and in screening of gastric cancer risks. J Gastrointest Disord Liver Funct *2*: 1-5, 2016. https://doi.org/10.15436/2471-0601.16.1242

99 Syrjänen K: Caveats in diagnosis of *Helicobacter pylori* infection can be avoided by a panel of serum biomarkers (GastroPanel®). An invited Editorial. J Carcinog Mutagen *7(6)*: e123. doi:10.4172/2157-2518.1000e123, 2016.

100 Syrjänen K: False negative and false-positive results in diagnosis of *Helicobacter pylori* infections can be avoided by a panel of serum biomarkers (GastroPanel®). M J Gast *1(1)*: 007-014, 2017. http://www.mathewsopenaccess.com/PDF/Gastroenterology/M_J_Gast_1_1_007.pdf

101 Benberin V, Bektayeva R, Karabayeva R, Lebedev A, Akemeyeva K, Paloheimo L and Syrjänen K: Prevalence of H. pylori infection and atrophic gastritis among symptomatic and dyspeptic adults in Kazakhstan. A hospital-based screening with a panel of serum biomarkers. Anticancer Res *33(10)*: 4595-4602, 2013. PMID: 24123036

102 Zagari RM, Rabitti S, Greenwood DC, Eusebi LH, Vestito A and Bazzoli F: Systematic review with meta-analysis: Diagnostic performance of the combination of pepsinogen, gastrin-17 and anti-*Helicobacter pylori* antibodies serum assays for the diagnosis of atrophic gastritis. Aliment Pharmacol Ther *46(7)*: 1-11, 2017. PMID: 28782119, DOI: 10.1111/apt.14248

103 Moayyedi P, Talley NJ, Fennerty MB and Vakil N: Can the clinical history distinguish between organic and functional dyspepsia? JAMA *295(13)*: 1566-1576, 2006. PMID: 16595759, DOI: 10.1001/jama.295.13.1566

104 Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM and Kuipers EJ: European *Helicobacter* Study Group. Management of *Helicobacter pylori* infection – the Maastricht IV/Florence Consensus Report. Gut *61(5)*: 646-664, 2012. PMID: 22491499, DOI: 10.1136/gutjnl-2012-302084

105 El-Serag HB, Kao JY, Kanwal F, Gilger M, LoVecchio F, Moss SF, Crowe S, Elfant A, Haas T, Hapke R and Graham DY: Houston Consensus Conference on Testing for *Helicobacter pylori* Infection in the United States. Clin Gastroenterol Hepatol *16(7)*: 992-1002. PMID: 29559361, DOI: 10.1016/j.cgh.2018.03.013

106 Pasechnikov VD, Chukov SZ, Kotelevets SM, Mostovov AN, Mernova VP and Polyakova MB: Invasive and non-invasive diagnosis of *Helicobacter pylori*-associated atrophic gastritis: A comparative study. Scand J Gastroenterol *40(3)*: 297-301, 2005. PMID: 15932170

107 Talley NJ, Vakil N, Delaney B, Marshall B, Bytzer P, Engstrand L, de Boer W, Jones R, Malfertheiner P and Agréus L: Management issues in dyspepsia: Current consensus and controversies. Scand J Gastroenterol *39(10)*: 913-918, 2004. PMID: 15513327, DOI: 10.1080/00365520410003452

108 Suovaniemi O: State of the Art GastroPanel and Acetium innovations for the Unmet Need. Biohithealthcare.com, 2011. Available at: https://biohithealthcare.com/wp-content/uploads/2018/12/state-of-the-art-gastropanel-and-acetium-innovations-suovaniemi-20111.pdf. Last accessed 28/01/2019.

109 Li Z, Wang G, Zhang S and Linghu E: Screening process for early gastric cancer in China (Draft 2017, Shanghai). Chin J Dig Endosc *35*: 1-13, 2018.

110 Tu H, Sun L, Dong X, Gong Y, Xu Q, Jing J, Bostick RM, Wu X and Yuan Y: A Serological biopsy using five stomach-specific circulating biomarkers for gastric cancer risk assessment: A multi-phase study. Am J Gastroenterol *112(5)*: 704-715, 2017. PMID: 28323271, DOI: 10.1038/ajg.2017.55

111 http://www.biohithealthcare.com/additional-information. Last accessed 28/01/2019.

*Received December 13, 2018*
*Revised January 27, 2019*
*Accepted January 28, 2019*