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Helicobacter pylori (Hp) IgG ELISA of the New-Generation GastroPanel® Is Highly Accurate in Diagnosis of Hp-Infection in Gastroscopy Referral Patients

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Abstract. *Background/Aim: Helicobacter pylori (Hp) infection affects a substantial proportion of the world population and is a major risk factor of gastric cancer (GC). The caveats of common Hp-tests can be evaded by a serological biomarker test (GastroPanel®, Biohit Oyj, Helsinki), the most comprehensive Hp-test on the market. The clinical validation of Helicobacter pylori IgG ELISA of the new-generation GastroPanel® test is reported. The aim of the study is to validate the clinical performance of the Helicobacter pylori IgG ELISA test in diagnosis of biopsy-confirmed Hp-infection in gastroscopy referral patients. Patients and Methods: A cohort of 101 patients (mean age=50.1 years) referred for gastroscopy at the outpatient Department of Gastroenterology (SM Clinic, St. Petersburg) were examined by two test versions to validate the new-generation GastroPanel®. All patients were examined by gastroscopy and biopsies, which were stained with Giemsa for specific identification of Hp in the antrum (A) and corpus (C). Results: Biopsy-confirmed Hp-infection was found in 64% of patients, most often confined to antrum. The overall agreement between Hp IgG ELISA and gastric biopsies in Hp-detection was 91% (95%CI=84.1-95.8%). Hp IgG ELISA diagnosed biopsy-confirmed Hp (A&C) with sensitivity (SE) of 92.3%, specificity (SP) of 88.6%, positive predictive value (PPV) of 93.8% and negative predictive value (NPV) of 86.1%, with AUC=0.904 (95%CI=0.842-0.967). In ROC analysis for Hp detection (A&C), Hp IgG ELISA shows AUC=0.978 (95%CI=0.956-1.000). Conclusion: The Hp IgG ELISA test successfully concludes the clinical validation process of the new-generation GastroPanel® test, which retains the unrivalled diagnostic performance of all its four biomarkers, extensively documented for the first-generation test in different clinical settings.*

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The important etiological role of *Helicobacter pylori* (Hp) in the development of gastric cancer (GC) has become increasingly clear since the first description of this bacteria in 1984 (1). According to the current understanding, GC develops from Hp-infection through a stepwise process of precancer lesions with increasing severity: mild, moderate and severe atrophic gastritis (AG), followed by intestinal metaplasia (IM) and dysplasia. This pathogenetic chain is known as the Correa cascade, involved in around 50% of all GC cases (2-11).

Similarly, also the management of Hp infection has undergone significant progress during the past decades, thanks to the major efforts made by the European Helicobacter Study Group starting from their first consensus conference of 1996 in Maastricht (12). Since then, these Maastricht conferences have been repeated every 4-5 years, each followed by a Consensus Report, the latest (2017) being the fifth in order (13). Attempts to standardize the diagnosis and treatment of Hp infections have led to several national guidelines as well (14, 15).

During the recent years, considerable attention has been paid to different tests available for diagnosis of Hp, also including comprehensive reviews of the advantages and limitations of their utility in different clinical settings (13-17). Unfortunately, this discussion has ignored many of the limitations of the commonly used Hp tests, although their shortcomings have been listed in all European Consensus Reports since 1996 (12, 13, 16, 17). This applies to both of the two most widely used Hp tests: the 13C-Urea Breath Test

(UBT) and Stool Antigen test (SAT), for which Barry Marshall (1) made an early warning already almost 25 years ago (18). 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 the UBT and SAT tests, false-negative (up to 40%) and false-positive results (UBT) being not uncommon (19-26).

The above listed caveats of the UBT and SAT tests (19-26) can be evaded by a serological biomarker test introduced by the Finnish biotechnology company Biohit Oyj (Helsinki, Finland) (21, 22). This serological (ELISA) test is based on four stomach-specific biomarkers: pepsinogen I (PGI), pepsinogen II (PGII), gastrin-17 (G-17) and Hp IgG antibodies (Hp IgG-ELISA), and known as GastroPanel® (27, 28). This biomarker panel is designed as the first-line diagnostic test for dyspeptic patients and for screening of the risk conditions of GC, *i.e.*, Hp-infection and AG (21, 22, 27-30).

Since its launch on the market in the early 2000's, GastroPanel® has attracted considerable global interest (13, 27, 31), extensively evaluated in a wide variety of clinical settings, and its outstanding characteristics were confirmed in two recent meta-analyses (32, 33). On the track towards GastroPanel® quick (POC) test, a new-generation (unified) GastroPanel® test has been launched, and recently underwent the first clinical validation in patients at high risk for AG (34). Because of an "incomplete study design", *i.e.*, biopsy verification of GastroPanel® test-positives only was allowed by the ethical committee (34), we were unable to validate the *Helicobacter pylori* IgG ELISA test of the new-generation GastroPanel® (34). In the present study, this was done for the first time in a series of gastroscopy-referral patients, all examined by gastroscopy and biopsies which were stained with Giemsa stain for specific identification of *Helicobacter pylori* in the biopsies (35).

Patients and Methods

Patients. As the clinical arm of this validation study of the new version of GastroPanel® test, the patients were enrolled at an outpatient Department of Gastroenterology, at SM-Clinic in St. Petersburg, Russia (an international provider of high-quality medical services, operating in 25 countries, *e.g.* Japan, China, Singapore and South-Korea: https://medica-tour.com/hospitals/treatment-in-russia/sm-clinic). Potentially eligible patients were identified among the consecutive outpatients referred for gastroscopy at SM-Clinic with different clinical indications. All patients were asked to participate in the study by signing a written consent. Eligible were considered all patients above 18 years of age, with or without upper abdominal symptoms. The following patients were considered non-eligible: 1) the patients whose treatment required surgery, or immediate follow-up treatment for major symptoms, as well as 2) those who refused to consent (35).

The study protocol followed the Declaration of Helsinki, and the whole trial was approved (June 2015) by the institutional review board of the SM-Clinic, fulfilling the rigorous ethical principles applied to all clinical studies in their hospitals worldwide (IRB-SM-StP: 06/2015). A cohort of 101 eligible patients completed the study protocol by the end of summer 2015. Of the 101 patients, 71 were women and 30 were men, with the mean age of 50.1 years (SD=16.7 years, as detailed in the Technical Report of this study (35).

Study design. This study is an open clinical trial using the new-generation GastroPanel® test to screen a cohort of gastroscopy-referral patients for the five diagnostic endpoints of the test: 1) healthy stomach, 2) superficial Hp-gastritis, 3) AG of the antrum; 4) AG of the corpus, and 5) AG of the antrum and corpus (pan-gastritis) (27-29, 30, 32, 34).

The new-generation (unified) GastroPanel® test. Prompted by the favorable feedback from the end users worldwide obtained by the original GastroPanel® test (Biohit Oyj, Helsinki, Finland)(10,27), in which all four biomarkers are being processed under different (incubation) conditions (27-29), Biohit R&D Department undertook a project to design the next generation GastroPanel® test in 2013. The goal was to develop a new assay, where all four biomarkers are being processed under unified conditions using an automatic ELISA instrument or manual processing. The new-generation (unified) GastroPanel® test contains the same four biomarkers as the original test version (28), maintaining its basic design as an ELISA test.

Because of the crucial technical modifications in the principal test components, the new-generation GastroPanel® test was treated as a novel test by registration authorities, which necessitates passing of all steps required for CE registration, including the validation studies for clinical performance. The first in order of these validation studies is reported here. Although conducted some years ago, the results of this study have never been published (due to the reasons to be discussed later), but only described in a Technical Report for official use (35).

Patient preparation for GastroPanel® sampling. After consenting to participate in the study, the patients were scheduled for an appointment to GastroPanel® testing at the laboratory of SM-Clinic, with written instructions for preparatory measures. Apart from the recommended 10-h fasting before sampling, the study subjects were instructed to discontinue their eventual PPI-medication, preferably one week before GastroPanel® sampling. If not possible due to intractable dyspeptic symptoms, a notice of PPI use had to be included in the GastroPanel® referral form, including the information whether PPI was interrupted or not, and for how many days (28-30).

Sample collection and processing. For consistent interpretation of the test results by the GastroSoft® application (Biohit Oyj), it is mandatory to complete the GastroPanel® referral form, as detailed before (28-30). A minimum of 2 ml EDTA plasma from a fasting blood sample was taken into an EDTA tube. Because not used for on-site testing, the EDTA plasma samples were frozen instantly (–70˚C). Using G-17 stabilizer (Biohit Oyj) (5% of the sample volume) enables a temporary storage in the refrigerator (at 2-8˚C), for up to 3 days, but immediate freezing at –70˚C was the preferred method of storage. This is most critical for G-17, to avoid decay at too high temperature (28-30, 32).

Stimulated G-17 (G-17s). Apart from the fasting sample for all 4 biomarkers, another blood sample is needed to measure the level of stimulated G-17 (G-17s) (28-30, 32), collected 20 min after intake of a protein drink with average protein content of 77%.

Helicobacter pylori IgG ELISA. *Helicobacter pylori* IgG ELISA is performed from the same plasma samples as the other markers (28). The test is based on an ELISA technique, with purified Hp bacterial antigen, adsorbed on a microplate, and a detection antibody labelled with horseradish peroxidase (HRP). The details of the test performance are given in the product IFU (instructions for use) (36). In brief, the assay proceeds according to the following reactions: 1) Partially purified *H. pylori* bacterial antigen attached to the polystyrene surface of the wells binds *H. pylori* IgG antibodies present in the sample; 2) Wells are washed to remove residual sample; 3) HRP-conjugated monoclonal anti-human IgG binds to the *H. pylori* IgG antibodies; 4) The wells are washed and the TMB substrate is added. The substrate is oxidized by the HRP enzyme, resulting in the formation of a blue end product; and 5) The enzyme reaction is terminated with the stop solution. *H. pylori*-positive samples turn yellow with calculated values of >30 EIU (enzyme immunounits), signifying a positive Hp-test (36).

GastroPanel® testing. The frozen plasma samples were delivered (by courier) from SM-Clinic (St. Petersburg) to the laboratory of Biohit Oyj (Helsinki) for analysis, using both the standard test version and the new-generation (unified) GastroPanel® test version (28, 29), following the instructions for use (IFU) of the test kits (36). To measure the test consistency, all analyses were conducted triplicates using two lots (1 and 2) of the new test version.

Evaluation of GastroPanel® results. The results of the GastroPanel® test were evaluated using the GastroSoft® software application. As repeatedly emphasized (28-30, 32, 39, 40), GastroPanel® test is optimized for use jointly with the Updated Sydney System (USS) classification of gastritis (5). Both include 5 diagnostic categories: a) normal mucosa, b) Hp-induced gastritis with no atrophy, c) atrophic gastritis of antrum (AGA), d) atrophic gastritis of corpus (AGC), and e) atrophic gastritis in both antrum and corpus (AGpan) (5, 28-30, 32, 39, 40), facilitating the reproducibility analyses (34).

Gastroscopy and biopsy procedures. As per the study design, all study subjects were enrolled among the consecutive patients referred for gastroscopy to SM-Clinic with different indications (symptomatic or asymptomatic). Accordingly, all 101 enrolled patients underwent gastroscopy and targeted biopsies. On gastroscopy, all observed abnormal mucosal lesions were noted and photographed, and if necessary subjected to additional biopsy. Endoscopic findings were classified into one of the following categories: 1) normal; 2) inflammation; 3) suspected atrophy; 4) definite atrophy; 5) ulcer; and 6) other abnormality.

Taking of gastric biopsies followed the USS protocol, with separate biopsies from the antrum and corpus (5). In each patient, routine biopsy specimens are taken from the antrum and corpus, at least two biopsies from each. Endoscopic biopsies were processed for microscopic examination using the routine procedures. In addition to conventional Haematoxylin and Eosin (HE), staining all biopsies were also stained with Giemsa stain for specific demonstration of *Helicobacter pylori* in the samples (1, 5, 10, 12-17).

All gastroscopy biopsies were examined by expert pathologists at the Department of Pathology, SM-Clinic and the diagnoses were classified using the USS classification of gastritis (5). The grade of AGA and AGC was graded into three categories (mild, moderate, severe), and in atrophic pan-gastritis (AGpan) both components (AGA, AGC) were graded separately. In Giemsa-stained sections, *Helicobacter pylori* were identified and quantified, using a semi-quantitative 4-tier grading: 0=absent; 1=Hp present in small quantities (=scanty); 2=Hp present in moderate quantities; and 3=Hp present in abundance (35). In some statistical testing, also a 3-tier grading was used, where grades 2 and 3 were combined.

Table I. *Distribution of the diagnostic categories tested by the two GastroPanel® versions*.

GastroPanel® profile	First-generation GastroPanel®		New unified GastroPanel®	
	No.	Per Cent	No.	Per Cent
Normal	33	33.0	34	34.0
Hp-gastritis	58	58.0	57	57.0
AGA	6	6.0	5	5.0
AGC	3	3.0	4	4.0
AGpan	0	0.0	0	0.0
Total No. Cases	100		100	

Weighted kappa (ICC)=0.968 (95%CI=0.953-0.979);
Overall concordance: 97/100=97.0 (95%CI=93.7-100)

AGA, Atrophic gastritis in the antrum; AGC, atrophic gastritis in the corpus; AGpan, atrophic gastritis in the antrum and in the corpus; ICC, intra-class correlation coefficient.

Statistical analyses. All statistical analyses were performed using the SPSS 26.0.0.1 for Windows (IBM, NY, USA) and STATA/SE 16.1 software (STATA Corp., Austin, TX, USA). The descriptive statistics was performed according to routine procedures. Performance indicators: sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV) and their 95%CI, of GastroPanel® Hp IgG ELISA (separately for antrum and corpus) were calculated using the algorithm introduced by Seed *et al*. (2001) (41). GastroPanel® is a quantitative ELISA test, and ROC (Receiver Operating Characteristics) curves were used to identify the optimal sensitivity/specificity balance for Hp IgG ELISA to detect biopsy-confirmed *Helicobacter pylori* infection. Significance of the difference between AUC values was estimated using the roccomb test. The agreement between i) GastroPanel® Hp IgG ELISA and ii) Hp-detection in the biopsies, as well as iii) between Hp IgG ELISA test lots, was calculated by using the conventional means for overall agreement (OA) as well as by regular (Cohen's kappa; 2×2 tables) and weighted kappa (κ_w) using the intra-class correlation coefficient (ICC) test, with the following defaults: parallel model, two-way random effects, absolute agreement. All tests were interpreted significant at the level of $p<0.05$. In addition, Fagan's nomogram was constructed to give the post-test predictions for Helicobacter detection by the Hp IgG ELISA at a population level, based on the test indicators calculated for the Hp-endpoint in this study by the STATA *diagti* algorithm (41): i) the pre-test probability; ii) positive likelihood ratio (LR+), and iii) negative likelihood ratio.

Results

Table I summarises the distribution of the five diagnostic profiles of the two GastroPanel® test versions. The two test

Table II. *Biomarker levels of the two lots of unified GastroPanel® across the five diagnostic categories.*

GP profile	PGI (M±SD)	PGII (M±SD)	PGI/PGII (M±SD)	G-17b (M±SD)	G-17s (M±SD)	HpAb (M±SD)
Normal						
Lot 1	85.3 (43.3)	10.1 (5.2)	8.6 (2.3)	6.2 (10.9)	14.3 (14.2)	14.8 (2.5)
Lot 2	82.4 (38.1)	10.0 (5.2)	8.5 (2.0)	6.8 (12.5)	16.2 (17.3)	16.6 (2.8)
Hp-Gastritis						
Lot 1	100.8 (43.8)	18.1 (11.4)	6.3 (2.4)	7.4 (8.7)	19.2 (13.1)	439.5 (457.4)
Lot 2	96.0 (41.2)	17.9 (11.2)	6.2 (2.4)	7.3 (8.4)	19.7 (13.2)	501.0 (536.9)
AGA						
Lot 1	73.2 (21.7)	13.6 (3.4)	5.4 (1.0)	1.2 (0.8)	1.4 (0.8)	228.8 (229.3)
Lot 2	71.2 (22.2)	13.8 (4.3)	5.2 (1.0)	1.3 (0.8)	1.4 (0.7)	283.8 (316.8)
AGC						
Lot 1	47.6 (23.8)	24.0 (13.2)	2.2 (0.9)	23.0 (16.0)	21.9 (16.2)	668.9 (434.5)
Lot 2	45.6 (22.8)	22.6 (11.4)	2.1 (0.6)	23.2 (16.2)	21.7 (16.2)	762.1 (590.7)
AGpan						
Lot 1	NA	NA	NA	NA	NA	NA
Lot 2	NA	NA	NA	NA	NA	NA
ICC	0.991 (95%CI= 0.979-0.995)	0.996 (95%CI= 0.993-0.997)	0.977 (95%CI= 0.966-0.985)	0.993 (95%CI= 0.990-0.995)	0.995 (95%CI= 0.992-0.997)	0.978 (95%CI= 0.965-0.986)

AGA, Atrophic gastritis in the antrum; AGC, atrophic gastritis in the corpus; AGpan, atrophic gastritis in the antrum and in the corpus; ICC (intra-class correlation coefficient) or weighted kappa between lot 1 and lot 2 across all diagnostic categories; NA, no cases available; GP, GastroPanel®; PGI, pepsinogen I; PGII, pepsinogen II; G-17, gastrin-17; HpAb, Hp IgG ELISA.

Table III. *Biomarker levels* across the five USS grades of gastritis.*

USS grade	PGI (M±SD)	PGII (M±SD)	PGI/PGII (M±SD)	G-17b (M±SD)	G-17s (M±SD)	HpAb (M±SD)	No. cases
Normal	82.3 (38.9)	9.5 (4.6)	8.9 (2.4)	5.7 (11.0)	12.2 (11.9)	18.2 (8.6)	35
Hp-gastritis	95.5 (39.5)	17.3 (10.1)	6.1 (1.9)	7.2 (7.4)	20.8 (13.8)	490.1 (511.2)	45
AGA	100.1 (49.2)	18.8 (9.6)	5.6 (1.9)	4.5 (4.0)	14.2 (15.5)	384.5 (418.3)	14
AGC	71.5 (59.3)	29.4 (17.3)	2.4 (0.8)	21.7 (14.3)	28.0 (18.2)	646.9 (464.7)	5
AGpan	NA	NA	NA	NA	NA	NA	0

*The two lots of unified GastroPanel test combined. AGA, Atrophic gastritis in the antrum; AGC, atrophic gastritis in the corpus; AGpan, atrophic gastritis in the antrum and in the corpus; USS, Updated Sydney System; PGI, pepsinogen I; PGII, pepsinogen II; G-17, Gastrin-17; HpAb, Hp IgG ELISA.

versions have practically identical performance, with an overall concordance of 97% (95%CI=93.7-100%), and weighted kappa (ICC) value of 0.968. These two figures implicate that the new test version retains all the technical characteristics of the previous GastroPanel® test version.

Table II summarizes the values (M±SD) of the 4 biomarkers, analysed by the two test lots of the unified GastroPanel® test, and stratified by the five diagnostic categories. As determined by the weighted kappa test, the results of the two test lots are practically identical for all four biomarkers and across the five diagnostic categories. This implicates a high degree of consistency, within a range of 0.977 to 0.996 (ICC).

Biomarker levels stratified by the five diagnostic categories of the USS classification are summarized in Table III. The biomarker levels in in the five diagnostic categories of the GastroPanel® and the USS are very similar. Interestingly, the highest titres of Hp IgG are found in AGC of both classifications.

Table IV illustrates the agreement between the new-generation GastroPanel® test and the USS classification. The overall agreement (OA) between the two tests is 0.808 (*i.e.*, 80.8%). Using the weighted kappa test (κw) to measure the agreement between GastroPanel® and the USS, κw=0.899 (95%CI=0.849-0.933).

The agreement between the Hp IgG ELISA test and the biopsy-confirmed detection of Hp in the antrum and corpus is illustrated in Table V. Altogether, 91/100 cases were concordantly diagnosed by IgG ELISA and gastric biopsies: OA=91.0% (95%CI=84.1-95.8%). The level of regular (Cohen's) kappa (κ=0.806) is also excellent, *i.e.*, an almost perfect agreement. The likelihood (OR) of diagnosing Hp in the biopsies after an Hp-positive IgG ELISA has OR in the range of 93 to 105 (p=0.0001).

Table IV. *Agreement between the unified GastroPanel® test and the USS classification.*

GastroPanel	The Updated Sydney System (USS)					Total
	Normal	HP-gastritis	AGA	AGC	AGpan	
Normal	31	3	0	0	0	34
HP-gastritis	4	41	10	1	0	56
AGA	0	1	4	0	0	5
AGC	0	0	0	4	0	4
AGpan	0	0	0	0	0	0
	Overall agreement (OA): 80/99; 0.808 (95%CI=0.730-0.885) *Weighted kappa (κ_w): ICC=0.899 (95%CI=0.849-0.933)					99

*Weighted kappa (ICC; parallel model, two-way random, absolute agreement); AGA, atrophic gastritis in the antrum; AGC, atrophic gastritis in the corpus; AGpan, atrophic gastritis in the antrum and in the corpus.

Table V. *Agreement between Hp IgG ELISA and biopsy-confirmed Hp-infection.*

GastroPanel® Hp IgG ELISA	Gastroscopic biopsy*		Total no. of cases
	Hp present**	Hp absent**	
Lot 1			
Hp positive	59	3	62
Hp negative	6	32	38
	Overall agreement (OA): 91/100; 0.900 (95%CI=0.841-0.958). (Cohen's kappa) κ=0.806 (95%CI=0.684-0.928) Odds ratio (OR)=104.9 (95%CI=24.7-447.7)		
Lot 2			
Hp positive	60	4	64
Hp negative	5	31	36
	Overall agreement (OA): 91/100; 0.900 (95%CI 0.841-0.9586). (Cohen's kappa) κ=0.803 (95%CI=0.679-0.927) Odds ratio (OR)=93.0 (95%CI=23.2-371.3)		
Lot 1 and 2 combined			
Hp positive	60	4	64
Hp negative	5	31	36
	Overall agreement (OA): 91/100; 0.900 (95%CI 0.841-0.9586). (Cohen's kappa) κ=0.803 (95%CI=0.679-0.927) Odds ratio (OR)=93.0 (95%CI=23.2-371.3)		

*Giemsa-staining for Helicobacter; **Hp present/absent in the antrum or in the corpus.

Table VI summarises the Hp IgG ELISA titres of the two lots of unified GastroPanel® stratified by the quantity of Hp present in the antrum and in the corpus, using the 4-tier (absent, scanty, moderate, abundant) grading. The two test lots give practically identical results (Pearson correlation: R2=0.973). Hp IgG ELISA titres show a better correlation with the quantified Hp in the antrum than in the corpus, where Hp is absent in the majority (56%) of biopsies. As to the antrum, the highest IgG ELISA titres are found in patients with moderate amount of Hp in the biopsies, in contrast to the corpus, where a scanty presence of Hp is accompanied by the highest IgG ELISA titres.

The indicators of Hp IgG ELISA test in diagnosis of biopsy-confirmed Hp-infection are summarised in Table VII, separately for the antrum, corpus or either of the two sites. The Hp IgG ELISA predicts the biopsy-confirmed Hp in the antrum with 93.8% SE and 88.9% SP, with AUC=0.913 (95%CI=0.853-0.973). For Hp present in the corpus, IgG ELISA shows even higher SE (95.3%) but substantially lower specificity (60.7%) and AUC=0.780 (for AUC difference; p=0.023).

Figure 1 illustrates the scatterplots where Hp IgG ELISA tires are plotted against the quantified Hp in the biopsies, using the 3-tier grading (absent, scanty, moderate/abundant). As to the antrum, the IgG ELISA titres increase in parallel with the increasing abundance of Hp in the biopsies (bivariate correlation: R^2=0.450; p=0.0001), whereas in the corpus, the IgG ELISA titres are highest in cases with scanty Hp in the biopsies (R^2=0.352, p=0.0001).

Figure 2 presents the ROC analysis for IgG ELISA (both lots combined) in detecting Hp in the biopsies of the antrum. The AUC value reaches an exceptional 0.980 (95%CI=0.958-1.000). The same ROC analysis for Hp-detection in the corpus is shown in Figure 3. Albeit less than for Hp in the antrum, the AUC value of 0.882 (95%CI=0.814-0.951) still falls within the highest category (0.8-1.0; almost perfect) of the performance tests. When Hp detection in either antrum or in corpus is taken into account, the AUC=0.978, with the 95%CI upper bound of 1.000 (Figure 4).

Figure 5 shows the Fagan's nomogram obtained by using the data derived from calculations of the Hp endpoint indicators in Table VII, *i.e.*, i) the pre-test probability 0.65; ii) positive likelihood ratio (LR+) 8.04, and iii) negative likelihood ratio (LR–) 0.086. The Fagan's nomogram gives the post-test predictions of Hp-detection, implicating that the Hp IgG ELISA of the unified GastroPanel® (30 EIU cut-off) predicts the detection of Hp with the likelihood of 94%. On the other hand, the likelihood is only 13% if the Hp IgG ELISA test result is negative for Hp.

Table VI. *Hp IgG ELISA levels* stratified by Hp-quantity in the biopsies.*

Hp quantified in	No of cases	Hp IgG Lot 1 (M±SD)	Hp IgG Lot 2 (M±SD)	Hp IgG Lot1/Lot 2 (M±SD)
Antrum				
Absent	36	17.3 (8.6)	18.9 (8.5)	18.1 (8.5)
Scanty	31	420.8 (395.3)	503.5 (513.0)	462.1 (451.3)
Moderate	17	598.7 (616.0)	641.7 (675.8)	620.3 (638.9)
Abundant	16	335.8 (302.1)	389.6 (373.8)	362.7 (337.2)
Significance	100	p=0.0001	p=0.0001	p=0.0001
Corpus				
Absent	56	121.6 (304.6)	140.7 (373.4)	131.2 (339.6)
Scanty	32	541.7 (461.7)	617.9 (505.1)	579.8 (476.7)
Moderate	8	433.0 (312.9)	483.9 (455.9)	458.4 (380.4)
Abundant	3	162.4 (191.7)	143.3 (157.1)	152.8 (174.3)
Significance	99	p=0.0001	p=0.0001	p=0.0001
			**Weighted kappa (κ_w): ICC=0.978 (95%CI=0.967-0.985)	

*Unified GastroPanel® Lot 1, Lot2 and both combined; **Weighted kappa (ICC; parallel model, two-way random, absolute agreement) between Lot 1 and Lot 2 across all categories.

Discussion

The new-generation GastroPanel® test has been clinically validated in two separate studies (34, 35). Both studies complement each other and collectively provide a formal validation of all 4 biomarkers of the new GastroPanel® test. The first of these validation studies (35), reported in this communication, is based on a "perfect study design", according to Reichenheim *et al.* (36), where all subjects examined with the diagnostic test of interest (GastroPanel®) were also confirmed by the gold-standard reference test (gastroscopy and biopsies). Unfortunately, however, the original goals of this validation study were not reached, because of the fact that the cohort of 101 gastroscopy-referral patients enrolled presented with an insufficient number of atrophic gastritis (AG) cases (n=14 AGA, n=5 AGC, n=0 AGpan) (Table III), thus precluding calculations of stable performance indicators for G-17 and PGI/PGII ratio in diagnosis of AGA and AGC, respectively (28-30, 32, 37-40).

Furthermore, as repeatedly emphasized, these indicators can be reliably calculated only by using the moderate/severe AGA/AGC (AGA2+/AGC2+) as the endpoint, because mild AGA and mild AGC are histological diagnosis that are poorly reproducible even among experienced pathologists, and thus a frequent cause of substantial verification bias in GastroPanel® studies (29, 30, 32, 33). In contrast to the verification bias caused by the "incomplete study design" (*i.e.*, only the study subjects testing positive with the diagnostic test of interest are being verified by the reference test), which can be corrected in part by statistical measures (36), the verification bias caused by misclassification of the reference test endpoints (AGA and AGC), cannot be corrected by any statistical technique. In the present cohort, only 2 cases of AGC2+ and 3 cases of AGA2+ are included, and because of this non-correctable limitation, the results of this first validation study were described in a technical report only (35).

In the second validation study targeted to patients at high risk for AG (34), a sufficient number of AGC2+ cases were enrolled, but the "incomplete study design" (34), hampered the calculation of stable estimates of sensitivity and specificity for the Hp IgG ELISA test in diagnosis of *Helicobacter pylori*, even if the statistical correction for this verification bias had been applied (36). Noteworthy, however, this failure to reach a sufficient number of AGA2+ and AGC2+ cases in the present cohort, does not compromise the validation of the Hp IgG ELISA test for Hp-infection, because all 101 patients were examined by gastroscopic biopsies, in which specific demonstration and quantification of *Helicobacter pylori* was possible in Giemsa-stained sections. Indeed, the results reported in this communication conclude the clinical validation process of the new-generation GastroPanel® test (34, 35), and fully confirm the outstanding performance characteristics of the new test version, fully compatible with the first-generation GastroPanel® test (28-30, 32, 33, 39, 40).

In the present cohort of 101 gastroscopy-referral patients, 64% (64/100; one missing data) patients tested Hp-positive (antrum or corpus) (Table V). This confirms the previously reported high prevalence of Hp-infections in Russian Federation, similarly as in other countries of the former Soviet Union, *e.g.* Kazakhstan (42, 43). In these high-risk countries, the Hp-prevalence of 60-70% exceeds the Hp-detection (slightly over 10% even in the high-risk age groups) in the low-risk countries by several folds (44). However, this high prevalence of Hp-infection makes the present cohort particularly suitable for the validation of the Hp IgG ELISA of the new GastroPanel®.

The consistency of the new GastroPanel® test is extremely high, as demonstrated by the almost perfect ICC between the two test lots, ranging from 0.977 to 0.996 (Table II). Similarly, the new test version also shows a high degree of agreement with the USS classification (Tables III and IV), for which the GastroPanel® test was originally optimised (28-30, 32, 33, 39, 40). This was also confirmed in the second part of the validation study among the high-risk patients, where enough AG cases were available to enable calculations of SE and SP for PGI and PGI/PGII ratio to diagnosis of AGC2+ (34). Although this was not possible in the present cohort, the excellent agreement between the new GastroPanel® and the USS was confirmed by calculating the overall agreement (OA) and weighted kappa (Table IV). Both these indicators favourably compete with the best

Table VII. *Hp IgG ELISA* in diagnosis of biopsy-confirmed Hp in the antrum and/or corpus.*

Hp+ Site	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	AUC (95%CI)
Antrum	93.8 (84.8-98.3)	88.9 (73.9-96.9)	93.8 (84.8-98.3)	88.9 (73.9-96.9)	0.913 (0.853-0.973)
Corpus	95.3 (84.2-99.4)	60.7 (46.8-73.5)	65.1 (52.0-76.7)	94.4 (81.3- 99.3)	0.780 (0 .708-0.852)
Antrum/Corpus	92.3 (83.0-97.5)	88.6 (73.3-96.8)	93.8 (84.8-98.3)	86.1 (70.5-95.3)	0.904 (0.842-0.967)
				**AUC comparisons:	
				A *vs*. C: p=0.023; C *vs*. AC: p=0.038; A *vs*. AC: p=0.859	

*Unified GastroPanel® Hp IgG ELISA with Lot1 and Lot2 combined; **Roccomp test between AUC values for antrum (A), corpus (C) and antrum/corpus (AC), *i.e*., Hp detection in the antrum, in the corpus, or in either of them; AUC, area under curve; PPV, positive predictive value; NPV, negative predictive value.

estimates ever reported in the large-scale international clinical studies (30).

The agreement between Hp IgG ELISA and Hp detection in the biopsies is outstanding (Table V). Both test lots gave concordant results with the gastric biopsies in 91/100 cases, when the subject was classified as Hp-positive if the bacteria were detected in the antrum or in corpus. This translates to OA of 91.0% (95%CI=84.1-95.8%), and regular (Cohen kappa) of 0.806 (95%CI=0.684-0.928). The likelihood of a subject testing Hp-carrier (Hp+) in the biopsy reaches OR of >100, when the GastroPanel® Hp IgG ELISA is Hp-positive (Table V). These ORs of 93-104.9 far exceed the ORs calculated for the likelihood of biopsy-confirmed AGC after GastroPanel® AGC profile in our second validation study (34).

The amount of Hp in the biopsies was quantified, and it was of interest to see, whether the Hp IgG ELISA titres bear any correlation to the quantity of the bacteria in the biopsies (Table VI, Figure 1). In this respect, antrum and corpus seem to be different. In the antrum, the increasing abundance of Hp seems to run almost in parallel with the level of Hp IgG ELISA titres (Table VI), particularly if the 3-tier grading is used (Figure 1) (p=0.0001; Pearson correlation). In the corpus, one cannot demonstrate a similar relationship, while the highest ELISA titres are found in cases where Hp is scanty, irrespective whether 4-tier or 3-tier grading is used (Table VI, Figure 1). This might have some bearing with the pathogenesis of Hp-infections in the stomach.

Hp-infection is considered to have its origin in the antrum and subsequently spreads to the corpus, if not successfully treated (5, 7-17). This process is protracted, taking years and even decades to progress from an acute Hp-infection to chronic AG (2-11). This would neatly explain why in the present cohort of patients, i) the prevalence of AG was quite low, and ii) why *Helicobacter pylori* infection was rarer in the corpus (n=43) than in the antrum (n=64). Most likely, these patients with 65% Hp-prevalence represent cases with relatively recent infection, confined to the antrum in most cases, and only gradually involving the corpus. Indeed, 33/64 (51.5%) Hp-positive cases in the antrum demonstrated moderate or abundant quantity of bacteria, as contrasted to only 11/43 (25.6%) of those in the corpus. This would explain why the subjects with Hp in the antrum have higher titres of Hp IgG ELISA antibodies and their closer correlation with the actual quantity of the bacteria in the biopsies (Figure 1, Table VI).

This different Hp-prevalence in the antrum and corpus also explains why the Hp IGG ELISA performs differently when Hp-antrum and Hp-corpus is used as the endpoint (Table VII). It is an established fact that with the declining prevalence, the PPV of the test will drop, also leading to decreased SP (37). This is exactly what happens to Hp IgG ELISA in diagnosis of Hp in the antrum and Hp in the corpus. Despite a similar test SE (around 94-95%) in both, the SP in the latter is almost 30% less (60.7% *vs*. 88.9%) than in the antrum. This results in a significantly (p=0.023) lower AUC values for the ROC curves of antrum (AUC=0.933) than those of corpus (AUC=0.780). Fortunately, however, the practical value of this observation in limited, because the patients are classified as Hp-positive irrespective whether the bacteria is present in the antrum or in the corpus (Table VII).

Given that GastroPanel® Hp IgG ELISA is a quantitative test, the conventional way of analysing the test performance is by ROC analysis, plotting quantitative ELISA titres (the test variable) against the state variable (Hp + or Hp-). The results of ROC analysis are also affected by the different Hp-prevalence in the antrum and corpus, although less dramatically (Figures 2-4) than in the above analyses using the 2×2 tables for SE and SP. In ROC analysis, Hp IgG ELISA detects biopsy-confirmed Hp in the antrum with AUC=0.980 (Figure 2), Hp in the corpus with AUC=0.882 (Figure 3), and Hp in either site with AUC=0.978 (Figure 4). AUC values of this range are exceptional for any diagnostic test, and those of Hp IgG ELISA even exceed the AUC values obtained for the other GastroPanel® biomarkers in diagnosis of AGC (34). The

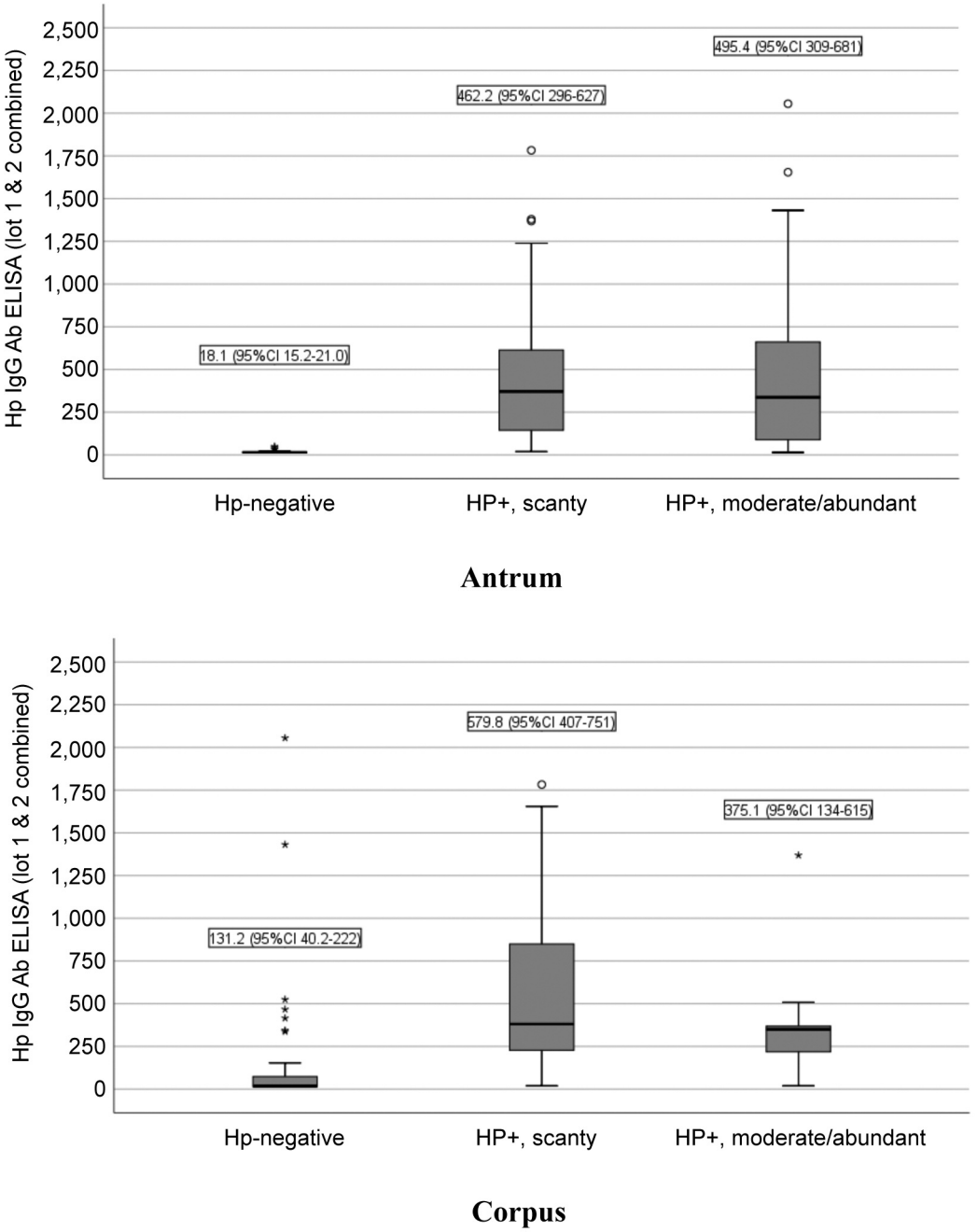

Figure 1. *Hp IgG ELISA titers scattered against quantified (3-tier grading) Helicobacter in the biopsies of the antrum and corpus.*

accuracy of this test is of completely different scale than the diagnostic precision of the commonly used Hp-tests (UBT and SAT), with their reported false-negative and false-positive error rates approaching 40% (19-26). These data on the formal validation of Hp IgG ELISA fully justifies the statements made in recent reviews, where GastroPanel® is recognised as the most comprehensive Helicobacter test (21, 22, 29, 30).

Importantly, the present results elaborated from a cohort of 101 patients are also generalizable at the population level, which is important because the population-based screening of GC risks (Hp and AG) is one of the two main indications of GastroPanel® use (28-30, 32, 33). This can be done using the approach described by Fagan in 1975 (45), subsequently known as the Fagan's nomogram (46-48). At present, the Fagan's nomogram is the simplest of the Bayes' theorem

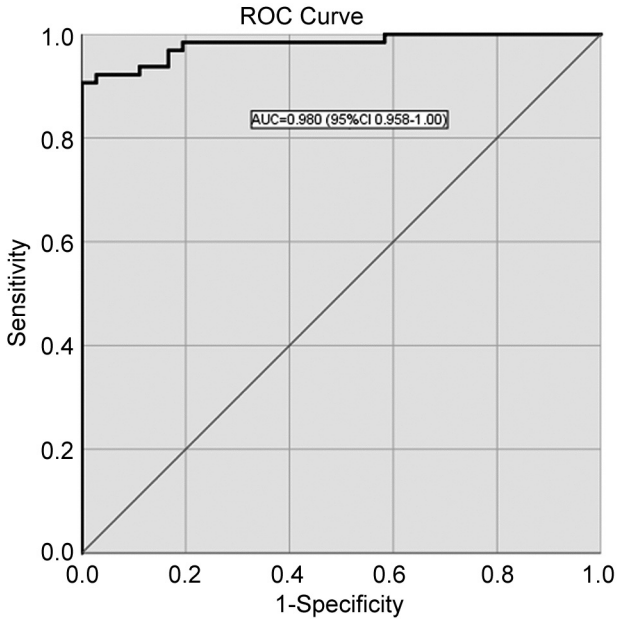

Figure 2. *Hp IgG ELISA* in detecting biopsy-confirmed Hp in the antrum by ROC analysis. *Lot 1 and Lot 2 Hp IgG ELISA.*

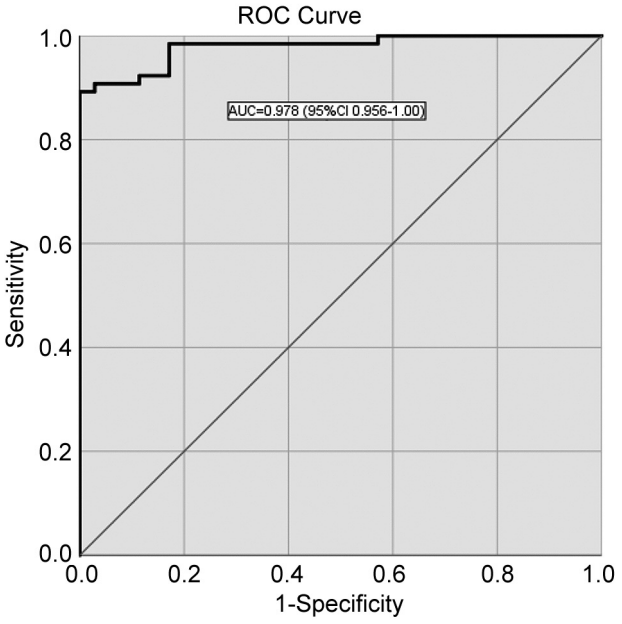

Figure 4. *Hp IgG ELISA* in detecting biopsy-confirmed Hp in the antrum/corpus by ROC analysis. *Lot 1 and Lot 2 Hp IgG ELISA.*

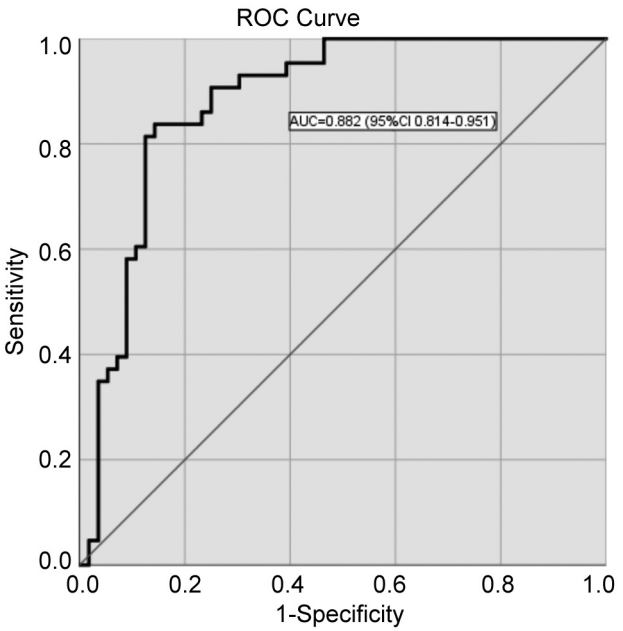

Figure 3. *Hp IgG ELISA* in detecting biopsy-confirmed Hp in the corpus by ROC analysis. *Lot 1 and Lot 2 Hp IgG ELISA.*

calculators to help practitioners determine the (post-test) probability of a patient truly having a condition of interest on the basis of a particular (pre)-test result (45-47). Assuming that the study samples are representative of the entire population, an estimate of the pre-test probability reflects the global prevalence of this disorder, and in this way, the likelihood ratios (LR+ and LR–) are clinically even more meaningful than SE and SP (47). When the likelihood ratios and the Hp-prevalence data generated from the calculations of test indicators by the algorithm of Seed et al (41) (Table VII) were applied, the Fagan's nomogram was obtained (Figure 5). The post-test predictions of Hp-infection in a population from which the present cohort was derived, implicate that a GastroPanel® Hp IgG ELISA predicts biopsy-confirmed Hp-infection with the likelihood of 94%, whereas this likelihood is only 13% if Hp IgG ELISA is negative.

Conclusion

The present validation study of the GastroPanel® Hp IgG ELISA test, based on a cohort of 100% biopsy-confirmed gastroscopy-referral patients, concludes the clinical validation process of the new-generation GastroPanel® test, because the other biomarkers (PGI, PGII, G-17) were validated in a separate study (34). These data confirm that in all respects, the new-generation GastroPanel® test retains the unrivalled diagnostic performance of the first-generation GastroPanel® test, which has been extensively characterised in different clinical settings worldwide since the test introduction in the early 2000's (30, 32, 33). This applies to the diagnosis of AG by the PGI, PGI/PGII, and G-17 biomarker profiles of the new test (34), as well as to the detection of biopsy-confirmed *Helicobacter pylori* infection,

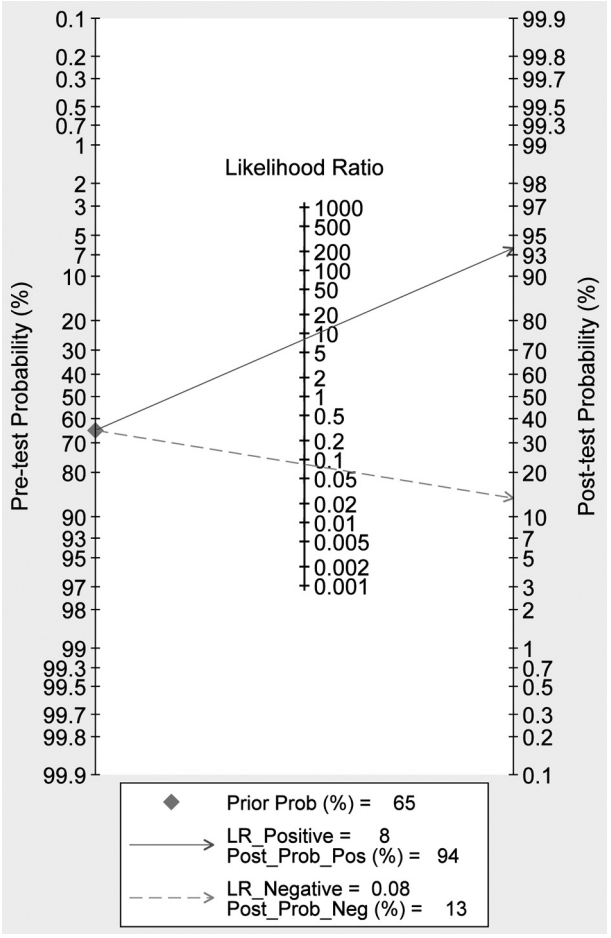

Figure 5. *Fagan's nomogram for Hp IgG ELISA as a predictor of Hp-infection at population level, constructed by using the pre-test probability, LR+ and LR– calculated in Table VII. LR+, positive likelihood ratio; LR–, negative likelihood ratio.*

firmly documented in the present study. The results are also directly transferrable to a population level. In any population with the prevalence of AG and Hp similar as in these two validation cohorts (34, 35), the new-generation GastroPanel® biomarker profile of AG or Hp shall predict these two risk conditions of GC with the likelihood of 94-95%, making GastroPanel® a perfect test for any population-based screening of gastric cancer risks worldwide (28, 30, 32, 33).

Conflicts of Interest

None to de declared.

Authors' Contributions

All Authors have met all the following four criteria: i) Substantial contributions to the conception or design of the work or the acquisition, analysis, or interpretation of data for the work. ii) Drafting of the work or revising it critically for important intellectual content. iii) Final approval of the version to be published. iv) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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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: 2711220. 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 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.

6 Filipe MI, Munoz N, Matko I, Kato I, Pompe-Kirn V and Jutersek A: Intestinal metaplasia types and the risk of gastric cancer: a cohort study in Slovenia. Int J Cancer *57(3):* 324-329, 1994. PMID: 8168991. DOI: 10.1002/ijc.2910570306

7 Correa P: A human model of gastric carcinogenesis. Cancer Res *48(13):* 3554-3560, 1988. PMID: 3288329.

8 Correa P: The epidemiology of gastric cancer. World J Surg *15(2):* 228-234, 1991. PMID: 2031359. DOI: 10.1007/BF01659057

9 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.

10 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(4):* 646-664, 2012. PMID: 22491499. DOI: 10.1136/gutjnl-2012-302084

11 Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S and Yamakido M: *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med *345(11):* 829-832, 2001. PMID: 11556306. DOI: 10.1056/NEJM200109133451111

12 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.

13 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

14 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

15 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

16 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.

17 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

18 Marshall B: The ^{14}C 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.

19 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

20 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

21 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, 2016. DOI: 10.4172/2157-2518.1000e123

22 Syrjänen K: False positive and false negative 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.

23 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

24 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

25 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.

26 Gisbert JP, Esteban C, Jimenez I and Moreno-Otero R: 13C-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

27 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

28 GastroPanel. Available at: http://www.gastropanel.com [Last accessed on August 31, 2020]

29 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

30 Syrjänen K, Eskelinen M, Peetsalu A, Sillakivi T, Sipponen P, Härkönen M, Paloheimo L, Mäki M, Tiusanen T, Suovaniemi O. DiMario F and Fan ZP: GastroPanel® Biomarker Panel: The most comprehensive test for *Helicobacter pylori* infection and its clinical sequelae. A critical review. Anticancer Res *39(3):* 1091-1104, 2019. PMID: 30842138. DOI: 10.21873/anticanres.13218

31 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

32 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

33 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(1):* 1-11, 2017. PMID: 28782119. DOI: 10.1111/apt.14248

34 Koivurova, O-P, Ukkola O, Koivikko M, Ebeling T, Yliaska I, Koskela R, Blomster T, Ala-Rämi A, Kettunen O, Karttunen TJ, Mäkinen M, Ronkainen J and Syrjänen K: Screening of the patients with autoimmune thyroid disease (AITD) and type 1 diabetes mellitus (DM1) for atrophic gastritis (AG) by serological biomarker testing (GastroPanel®). EC Gastroenterol Digest Syst *7(8):* 181-195, 2020. https://www.ecronicon.com/ecgds/pdf/ECGDS-07-00621.pdf

35 GastroPanel (unified) validation. Technical Report. Available at: https://www.biohithealthcare.com/wp-content/uploads/2020/08/gp-cli-report.pdf [Last accessed on August 31, 2020]

36 GastroPAnel Hp IgG ELISA IFU (instructions for use). Available at: https://www.biohithealthcare.com/wp-content/uploads/2018/12/hp-igg-ifu-multilanguage1.pdf [Last accessed on August 31, 2020]

37 Reichenheim ME and Ponce de Leon A: Estimation of sensitivity and specificity arising from validity studies with incomplete designs. The Stata J *2(1):* 267-279, 2002. DOI: 10.1177/1536867X0200200303

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 Syst *1(6):* 209-222, 2017.

39 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):* 1-10, 2017. DOI: 10.21767/2472-1646.100024

40 Syrjänen K: Serum biomarker panel (GastroPanel®) and slow-release L-cysteine (Acetium® Capsule): Rationale for the primary prevention of gastric cancer. EC Gastroenterol Digest Syst *3(6):* 172-192, 2017.

41 Seed PT and Tobias A: Summary statistics for diagnostic tests. Stata Techn Bull *59(1):* 9-12, 2001.

42 Roman LD, Lukyanchuk R, Sablin OA, Araslanova EI, Eklund C, Hendolin P, Paloheimo L and Syrjänen K: Prevalence of infection and atrophic gastritis in a population-based screening with serum biomarker panel (GastroPanel®) in St. Petersburg. Anticancer Res *36(8):* 4129-4138, 2016. PMID: 27466521.

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

44 Aine R, Kahar E, Aitokari K, Salminen J, Eklund C, Paloheimo L, Peetsalu A and Syrjänen K: Atrophic gastritis (AG) and its clinical sequels among elderly people in Finland and Estonia. A comparative study using GastroPanel and B12-vitamin testing of the residents in assisted-housing facilities. J Aging Res Clin Pract *5(4):* 194-202, 2016. DOI: 10.14283/jarcp.2016.115

45 Fagan TJ: Letter: nomogram for Bayes theorem. N Engl J Med *293(5):* 257, 1975. PMID: 1143310. DOI: 10.1056/NEJM197507312930513

46 Reid M, Lane DA and Feinstein AR: Academic calculations *versus* clinical judgments: practicing physicians' use of quantitative measures of test accuracy. Am J Med *104(4):* 374-380, 1998. PMID: 9576412. DOI: 10.1016/s0002-9343(98)00054-0

47 Van den Ende J, Moreira J, Basinga P and Bisolfi Z: The trouble with likelihood ratios. Lancet *366(9485):* 548, 2005. PMID: 16099289. DOI: 10.1016/S0140-6736(05)67096-1

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