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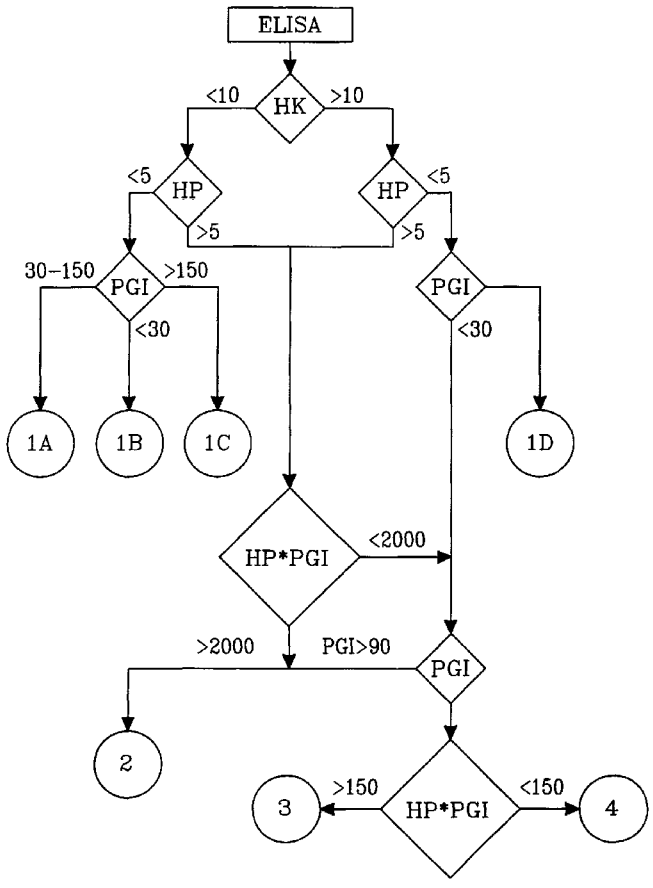

(57) Abstract: The present invention relates to a method for diagnosing possible presence of gastritis in a human by evaluating a blood sample, comprising assaying the blood sample for the presence of antibodies specific for H,K-ATPase, antibodies specific for Helicobacter pylori, and the concentration of pepsinogen I, whereby the presence of H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I concentration are compared between themselves and in relation to the respective values of H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen concentration of a normal population, in a software related system, wherein altered levels in the sample is indicative of gastritis, and whereby, preferably, an altered level detection leads to the issuance of a remittance for further investigation with regard to gastritis.

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TITLE
SCREENING METHOD FOR GASTRITIS

DESCRIPTION

BACKGROUND OF THE INVENTION

Dyspepsia, or indigestion, is a common diagnosis in primary health care, but with poorly defined management. The annual prevalence of dyspepsia in the United Kingdom (UK) is about 25%, and in primary health care it accounts for 3-4%.of the consultations (Harris, A., Eur J Gastroenterol Hepat 1999; 11 (Suppl I): S31-5). Among the chronic disorders of the upper gastrointestinal tract are those which fall under the general category of gastritis. Gastritis is an inflammation of the stomach mucosa which is manifested by a broad range of poorly-defined symptoms such as indigestion, "heart burn" and excessive eructation. The typical means used to diagnose gastrointestinal disorders depends on such factors as the nature and severity of symptoms, the overall health of the individual, the medical history of the patient, the need for a specific diagnosis in order to implement a treatment with reasonable likelihood of success, and the availability of diagnostic devices.

Esophagogastroduodenoscopy (EGD) with histopathological examination of biopsies is the gold standard to determine the status of the gastric and duodenal mucosa. This examination is safe, accurate and sometimes indispensable, e.g., in the older age group and especially in the presence. of alarm symptoms such as weight loss, anorexia, dysphagia, or gastrointestinal blood loss. The demand for upper endoscopy is increasing and in the UK approximately 0.5% of the population undergo this examination each year (Working Party of the Clinical Services Committee of the British Society of Gastroenterology, Provision of gastrointestinal endoscopy and related services for a district general hospital. Gut 1991; 32: 95-105; Gear, M.W.L., and Wilkinson, S.P., Br J Hosp Med 1989; 41: 438-44). Without EGD and visual inspection of the mucosa, gastritis is difficult to diagnose. However, EGD is expensive, inconvenient for the patient, and generally not recommended for children or patients with severe cardiopulmonary disease. Thus, for patients not having severe symptoms, a precise diagnosis of a gastrointestinal disorder might not be attempted. Such patients may simply be treated with conventional therapies, such as with antacids or drugs which inhibit stomach acid secretion. While such therapies might provide temporary relief of the symptoms, a cure is not often achieved. More effective treatments generally depend on a better diagnosis of the actual underlying gastrointestinal disorder. For example, many gastrointestinal disorders are mediated by bacterial infection of the mucosa, in

which case treatment of the bacterial infection would most likely be required to effectively treat the manifested gastrointestinal disorder.

There is a need for a simple pre-gastroscopic screening method to reduce the endoscopy workload, and attempts have been made in this direction with some success in patients with uncomplicated simple dyspepsia (Bodger, K., et al., Scand J Gastroenterol 1999; 34: 856-63; and Moayyedi, R, et al., Eur J Gastroenterol Hepatol 1999; 11:1245-50). In young dyspeptic patients (< 40 years) screening for Helicobacter pylorl (H. pylori) infection and a treatment strategy based on the presence of an infection reduces the endoscopy workload. This strategy appears as effective as an endoscopy-based strategy in reducing dyspeptic symptoms, dyspepsia consultation rates and the prescription of anti-secretory drugs (Moayyedi, P., et al., Eur J Gastroenterol Hepatol 1999; 11:1245-50). In the elderly with dyspepsia, however, the prevalence of gastritis and its consequences are considerably higher pointing at EGID as the initial diagnostic step.

The inflamed gastric mucosa transmits specific information to the blood stream that allows diagnosis of gastritis by serologic analysis. The morphology and cellular composition of the mucosa vary between the acid secreting corpus and the antrum. This may aid to distinguish corpus and pangastritis from antral gastritis. A number of serological markers have been described. Infection with H. pylori is the major cause of chronic gastritis, duodenal ulcer, mucosa associated lymphoid tissue (MALT) lymphoma and gastric cancer (Chiba, N., et al., Can Fam Physician 1998.; 44: 1481 -8; Genta, R.M., Gut 1998; 43: 35-8; Coyle, W.J., et al., Gastrointest Endosc 1998; 48: 327-8; Lee, B.M., et al., Jpn J Cancer Res 1998; 89: 597-603), and antibodies to various H. pylori antigens can easily be detected in 'the blood (Bodger, K, et al., Scand J Gastroenterol 1999; 34: 856-63; Moayyedi, P., et al., Eur J Gastroenterol Hepatol 1999; 11:1245-50). This infection is sometimes associated with an autoimmune reaction leading to atrophy of the corpus mucosa (Ozasa, K, et al., Dig Dis Sci 1999; 44: 253-6). A common feature of gastric autoimmunity and frequently several other autoimmune diseases, e.g., thyroiditis, insulin dependent diabetes mellitus and sometimes rheumatoid arthritis, is the occurrence of parietal cell autoantibodies (Bech, K, et al., Acta Endocrinol.1 991, 124: 534-9; Barrio, R., et al., Pediatr Endocrinol Metab 1997, 10: 511-6; Datta, A., et al., Indian J Med Res 1990, 92: 228-32; Mårdh, S., et al., Scand J Gastroenterol 1991, 26: 1089-96). The parietal cell H,K-ATPase a- and R-subunits were found to be the major autoantigens in autoimmune atrophic gastritis (Karlsson, A., et al., J Clin Invest 1988, 81A75-9; Song, Y.H., et al., Scand J Gastroenterol 1994, 29:122-7; Ma, J.Y., et al., Scand J Gastroenterol 1994, 20: 790-4). A low titre of H,K-ATPase antibodies is normally found in healthy individuals due to the

normal turn-over of parietal cells. In patients with inflamed corpus mucosa the titre may be increased.

Pepsinogen 1 (PG[) is secreted by the chief and mucous neck cells of the corpus mucosa into the lumen of the stomach but a small fraction (about 1 %) leaks into the blood stream (Baron, JR, Clinical tests of gastric secretion: History,' Methodology and Interpretation. (1978) London: Macmillan). Increased serum concentrations of PG1 are frequently found in patients With duodena) ulcer (Samloff, I.M., et al., Gastroenterol 1975 Jul, 69(1). 83-90). In patients with pernicious anaemia due to severe atrophy of the corpus mucosa serum PG1 is significantly reduced (Samloff, I.M., et al., Gastroenterol 1982 Jul, 83(1 Pt 2): 204-9).

Existing, non-invasive methods of detecting gastrointestinal disorders include monitoring blood flow to the affected region to detect inflammation (US 5;524,622). A significant disadvantage of this method is the requirement of injecting multiple substances into the patient followed by the detection by gamma camera. Additionally, the method only detects inflammation, and does not address the underlying cause of any inflammation. Other methods of detecting gastrointestinal disorders include assays for individual analytes such as pepsinogen (US 5,879,897) or Helicobacter pylori (US 5,814,455; 6,067,989;6,068,985; 6,090,611). Other serological markers are gastrin (Borch, K, et al., Scand J Gastroenterol 1997, 32:198-202), pepsinogen 11 (Carmel, R., Am J Pathol 1998, 90: 442-5), intrinsic factor antibodies (Waters, H.M., et al., J Clin Pathol 1989, 42: 307-12) and pepsinogen antibodies (Mårdh, S., et al., Acta Physiol Scand 1989, 136: 581-7). Although each one of these markers may be used to diagnose changes in the gastric mucosa, the overlap between healthy subjects and patients is great, and even greater among the various subgroups of patients. Therefore none of all these markers alone is sufficient for a reliable diagnosis.

SUMMARY OF THE INVENTION

The instant invention provides a screening method for gastritis in its various forms involving the evaluation of assay results for H,K-ATPase antibodies, H. pylori antibodies, and serum pepsinogen 1 concentration. The analysis of multiple analytes associated with gastritis provides a reliable indication of various subgroups of gastritis.

In one embodiment the invention comprises a method for diagnosing possible presence of gastritis in a human by evaluating a blood sample, comprising assaying the blood sample for the presence of antibodies specific for H,K-ATPase, antibodies specific for Helicobacter pylori, and the concentration of pepsinogen I, whereby the presence of

H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I concentration are compared between themselves and in relation to the respective values of H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen concentration of a normal population, in a software related system, wherein altered levels in the sample is indicative of gastritis, and whereby, preferably, an altered level detection leads to the issuance of a remittance for further investigation with regard to gastritis.

A preferred embodiment relates to a method, wherein the step of determining the levels of said indicators comprising performing immunoassays for detecting the indicators.

A another preferred embodiment relates to a method, wherein the group of indicators includes an additional indicator comprising the level of pepsinogen I multiplied by the level of Helicobacter pylori antibodies, and wherein the level of this additional indicator is compared to a standard.

A further other preferred embodiment relates to a method, wherein the levels of H,K-ATPase antibodies and Helicobacter pylori antibodies, which are significantly higher than the levels in a normal population is indicative of gastritis.

A further preferred embodiment relates to a method, wherein a lowered level of pepsinogen I concentration is indicative of corpus atrophy.

A preferred other embodiment relates to a method, wherein an increased level of pepsinogen I concentration is indicative of a corpus gastritis, optionally without any autoimmunity involved.

A further other preferred embodiment relates to a method, wherein the level of H,K-ATPase antibodies differing from that of the normal population is indicative of an autoimmune corpus atrophy.

A another preferred embodiment relates to a method, wherein the level of Helicobacter pylori antibodies differing from that of the normal population is indicative of antrum, or pangastritis.

A further preferred embodiment relates to a method, wherein increased levels of Helicobacter pylori antibodies, and normal to lowered concentrations of pepsinogen I is indicative of atrophy.

A preferred other embodiment relates to a method, wherein very low concentrations of pepsinogen I in combination with increased levels of H,K-ATPase antibodies is indicative of corpus atrophy.

A further aspect of the invention relates to a kit for screening for gastritis comprising reagents suitable for detecting H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I concentration.

A preferred embodiment of this latter aspect is a kit, wherein the reagents comprise pepsinogen I antibodies, H,K-ATPase and Helicobacter pylori proteins or peptides thereof.

A preferred other embodiment of this latter aspect is a kit, wherein the reagents comprise pepsinogen I, H,K-ATPase and Helicobacter pylori antigens immobilized on a solid support.

A another preferred embodiment of this latter aspect is a kit, wherein it further comprises labelled anti-human antibodies.

A further preferred embodiment of this latter aspect is a kit, wherein the reagents are provided in amounts sufficient to perform substantially equal numbers of assays to detect H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I concentration.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1 A-1 D are graphs showing the levels of H,K-ATPase antibodies, H. pylori antibodies, pepsinogen 1, and result of H. pylori antibody level multiplied by pepsinogen 1 concentration, respectively, for individuals with normal gastric mucosa (N) and those with duodenal ulcer (DU), atrophic gastritis (AG) and gastritis with pernicious anaemia (PA).

FIG. 2 is a flow chart depicting a testing evaluation scheme.

FIGS. 3A-3D are pie charts showing the four major groups identified by the screening tests of the instant invention for the test groups N, DU, AG, and PA.

FIGS. 4A-4D are pie charts showing the four major groups identified by the screening tests of the instant invention for the general population.

DETAILED DESCRIPTION OF THE INVENTION

Gastritis and dyspepsia are common entitles in primary health care but with poorly defined management. The aim of the present invention is to provide a serologic screening test for gastritis. Esophagogastroduodenoscopy (EGD) with biopsy and histological examination requires a skilful and experienced staff and it is presently the only reliable technique for diagnosing gastritis, benign ulcer and neoplasia. The last two groups are generally closely associated with chronic gastritis. Previous investigations often concluded that serology has a limited diagnostic value. However, the instant evaluation scheme for analyzing serology data is useful as a pre-gastroscopic screening of dyspepsia, irrespective of the age of the patient, which is a remarkable improvement compared with previous reports (Bodger, K, et al., Scand J Gastroenterol 1999, 34: 856-63; Moayyedi, R, et al., Eur J Gastroenterol Hepatol 1999, 11:1245-50). The immune system and chemical signalling from the inflamed gastric mucosa provide serum analytes and diagnostic possibilities for detection of gastritis.

Assay methods for determining the level of H,K-ATPase antibodies, Helicobacter pylori antibodies and pepsinogen 1 concentration are known. In a preferred embodiment of the instant invention, the method of determining the level of the analytes is by immunoassay. The immunoassay may be any of the well-known methods, including, but not limited to, enzyme-linked-immunosorbent assays (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), immunoprecipitation (IP),' and optical or electrochemical detection of immunoligand interaction. In a preferred embodiment, the immunoassay is one in which the antigen is immobilized on a solid support, samples added, followed by labelled antibody.

One embodiment of the invention is a kit for screening for gastritis. The kit comprises the reagents required to perform assays for at least two indicators selected from the group consisting of H,K-ATPase antibodies, Helicobacter pylori antibodies, and the level of pepsinogen 1. In a preferred embodiment, the assays are immunoassays, and the kit comprises analytes immobilized on one or more solid supports, such as a microtitre plate, strip of paper, nitrocellulose or other suitable material. Labelled antibody is included for detection. The kit preferably contains reagents for each assay in amounts sufficient to perform equal numbers, of the different assays. For example, a kit may contain sufficient reagents to perform ten H,K-ATPase assays, ten Helicobacter pylori assays and ten pepsinogen assays. The kit may additionally comprise analyte-specific antibodies, labelling reagents, positive and negative controls and wash solutions.

The immunoassay methods are based on analyses of a blood sample (or plasma or serum) from patients; the autoantibodies against H,K-ATPase, antibodies against Helicobacter pylori and concentration of pepsinogen are assayed. All of these analytes serve as markers of an inflammatory condition in the gastric mucosa. The methods of detecting the analytes, such as immunoassays, are well-known in the art.,

The assay results are analysed by a new grouping procedure in which the results are compared with reference values from healthy individuals from the normal population. A mathematical expression (the product of pepsinogen concentration multiplied by the titre of Helicobacter pylori antibodies) is essential to identify one particular group of patients. Other mathematical procedures may be used provided they achieve determination of a useful grouping of gastritis patients. This grouping procedure diagnoses inflammatory conditions in the gastric mucosa that previously have required the more costly and complicated gastroscopy with histopathological examination of biopsies from the mucosa.

The reference values from healthy individuals from the normal population may be standardized for each assay. In this embodiment, the test results are compared to standardized reference values in order to determine the patient's likelihood of having gastritis. A kit would contain a list of the standardized reference values for each assay. In an alternative embodiment, the reference values are obtained by testing a normal control along with the patient samples. In this embodiment, a kit would contain a normal control and standards. When performing the assays, one would run the assay on the control and standards at the same time the patient samples are tested. The assay results for the control and standards would then be compared to the results for the patient samples.

Sera from subjects examined with gastroscopy and biopsy were analysed for H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen 1. The diagnoses were normal gastric mucosa (n=50), duodenal ulcer (n=53), and atrophic corpus gastritis with (n=50) or without pernicious anaemia (n=46). An evaluation scheme (flow chart) was constructed to optimise the diagnostic agreement between serology and gastric mucosal morphology. Four major serologic groups and thirteen subgroups were obtained with an over-all sensitivity to detect gastritis of 98% (146/149) (95% CI 94-100%) and a specificity of 84% (42/50) (95% CI 71-93%). Additional sera from 483 subjects from the general population were grouped by serology. The overall sensitivity to detect gastritis in this population was 88% (211/240) (95% CI 83-92%) with a specificity of 81% (196/243) (95% CI 75-85%), There was a good, agreement between serology and the gastric mucosal morphology both in the groups used for developing the evaluation

scheme and in the sample of the general population. Thus, serology is appropriate for initial identification of subjects with a normal gastric mucosa, those who qualify for eradication of Helicobacter pylori, and those who are at risk of developing malignancy and therefore require gastroscopic examination.

In reading the assay results, altered levels of the analytes in a patient sample as compared to normal control values is indicative of the patient having gastritis. By Raltered" is meant levels either significantly above or significantly below the levels of the normal control. What is significant depends on the accuracy and precision of the specific test performed and may be determined empirically without undue experimentation. Additionally, the levels of multiple analytes in a patient sample are compared to normal control values in order to obtain a more accurate determination of whether or not the patient has gastritis. For example, in general, patient levels of H,K-ATPase antibodies and Helicobacter pylori antibodies above normal control levels is indicative of gastritis, while patient levels of the pepsinogen concentration either significantly above (duodenal ulcer) or below (severe atrophy of the corpus mucosa) normal control levels may be indicative of gastritis. The pepsinogen concentration multiplied by the Helicobacter pylori antibody titre either significantly above or significantly below the normal control may be indicative of gastritis. In order to determine the likelihood of the patient having gastritis and/or to determine the subgroup of the patient's gastritis, the combination of multiple assay results are compared to normal control values. Because some analytes may be either higher or lower than normal control levels and still be indicative of gastritis, the comparison of levels of multiple analytes in a patient sample to normal control levels of the same analytes provides a more accurate determination of gastritis.

With histomorphological examination as the gold standard, the present invention demonstrates that a combination of serologic assays detects 87% (196/225, group 1A in FIG, 2; Table 2) *of* subjects with normal gastric mucosa in a sample of the general population and in the study groups it was 84% (42/50, group 1A; Table 1). In the study groups, serology detected 91 % (48/53, group 2) of subjects with duodenal ulcer and 84% (81196, groups 3 plus 4) *of* subjects with corpus predominant atrophic gastritis with or without pernicious anaemia. All subjects with pernicious anaemia were obtained in group 4. In the population sample,, groups 2A-D comprised 15%. Among these, only 3% had a normal mucosa, while antral gastritis with atrophy and pangastritis overall comprised 88%. All subjects in groups 2A-D were H. pylori-positive. It is therefore suggested that subjects aged 40-50 years or less (Bodger, K, et al., Scand J Gastroenterol 1999, 34: 856-63; Moayyedi, R, et al., Eur J Gastroenterol Hepatol 1999,

11 *1245-50) that are obtained in groups 2A-D may be treated according to the current recommendations for peptic ulcer disease.

Only 12 subjects in the population sample were obtained in group 4A4C; one had non-atrophic corpus predominant gastritis and the remaining 11 atrophic corpus predominant gastritis. Thus, subjects belonging to group 4 should be recommended EGID due to the increased risk of malignancy.

In the population sample there were 12% (29/225) with normal gastric mucosa, but abnormal results of the serological analysis. There are probably several explanations for this discrepancy; some results of serum analytes may reflect a previous inflammatory condition, or the ELISAs may be more sensitive than the most experienced examiner to detect small changes in the mucosa.

In conclusion, EGID with biopsy remains the gold standard for an accurate diagnosis of the status of the gastric mucosa. However, as the following examples show, serology is a good complement when combined with the patient's symptomatology and medical history. It functions as a "serologic biopsy". Therefore, serologic assays are well-suited for pre-gastroscopic screening of dyspepsia to identify: (a) patients with normal gastric mucosa (group 1A), (b) H. pylori-positive patients with high levels of serum PG1 and high "H.p. x PG1 -factor" (group 2), and (c) patients with corpus predominant atrophic gastritis (groups 3 plus 4). Patients in (a) may be examined further for "non-acid-related" disorders, those in (b) younger than 40-50 years may be treated according to the recommendations for peptic ulcer disease, and in cases with unsuccessful treatment, the patients should be referred to EGD. Patients in (c) are at a higher risk of developing gastric malignancy and should therefore be referred to EGD.

The described serologic assays and the evaluation procedure are simple and may be performed in any clinical laboratory with some experience in immunoassays. They provide a reduction in the endoscopic workload, are beneficial for the patient, provide a valuable diagnostic too) for the doctor and are cost-efficient.

EXAMPLE 1

For the initial evaluation of the serologic results, sera from four groups of subjects examined endoscopically and histologically were selected; 50 subjects with normal gastric mucosa (N) (38 males and 12 females, median age 63 years, range 3780), 53 subjects with acute duodenal ulcer (DU) (39 males and 14 females, median age 52 years, range 20-79), 46 subjects diagnosed as having mild to severe corpus , predominant atrophic gastritis (AG) (23 males and 23 females, median age 68 years,

range 40-82), and 50 subjects with corpus predominant atrophic gastritis with pernicious anaemia (PA) (23 males and 27 females, median age 68 years, range 4083). The criteria for the diagnosis of pernicious anaemia which included a Schilling test showing intrinsic factor deficiency have previously been given (Borch, K, et al., Scand J Gastroenterol 1984, 19: 154-60).

EXAMPLE 2

A sample of 483 subjects (266 males and 217 females, median age 65, range 37 to 85 years) randomly selected from a general population in Sweden was examined with EW with biopsy and blood sampling. Results of this study have recently been published (Borch, K, et al., Dig Dis Sci, 2000, 45: 1322-29). In biopsy specimens, gastritis was classified according to the Sydney system into antrum predominant-, corpus predominant- and pangastritis with or without atrophy and with or without presence of H. pylori (Price, A., J Gastroenterol Hepatol 1991, 6: 209-22; Dixon, M.F., et al., Am J Surg Pathol 1996, 20: 1161-81). EW was performed as previously described (Borch, K, et al., Dig Dis Sci, 2000, 45: 1322-29). Three biopsies were taken from the gastric body (major-, anterior-, and posterior aspect), and the antrum within 3 cm of the pylorus.

EXAMPLE 3

Preparation of antigens

H,K-ATPase was prepared from pig gastric mucosa as previously described (Mårdh, S., et al., Scand J Gastroenterol 1991, 26: 1089-96). The binding of autoantibodies against the H,K-ATPase in this porcine antigen preparation was similar to that of the human antigen (Song, Y.H., et al., Scand J Gastroenterol 1994, 29: 122-7; Ma, J1.Y., et al., Scand J Gastroenterol 1994, 20: 7904; Karlsson, F.A., et a)., Clin exp Immunol 1987, 70:604-10). The vesicular membranes enriched with H,K-ATPase were treated at a low concentration of detergent (0.13% (w/v) of n-octylglucoside, or 0.06% (wlv) of sodium dodecylsulfate) to remove loosely attached proteins, e.g., pepsin/pepsinogen, and then stored at -70T in sucrose/Hepes-Tris buffer, pH 7.4. Antigens of H, pylori were prepared from five strains (CCUG 17874, 25, 66, 1139 and 253) as described by Ma et al. (Ma, J.Y., et al., Scand J Gastroenterol 1994, 29: 961 6).

EXAMPLE 4

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed essentially as described (Ma, J.Y., et al., Scand J Gastroenterol 1994, 29: 961-6) using Nunc-Immuno®) plates (Maxisorp®, Nunc, Roskilde, Denmark) coated with 50 pi of indicated antigen preparations (5 pg/ mi) in 50 mM sodium carbonate buffer, pH 9.8, and incubated overnight at 40C. The wells were

sequentially incubated with sera diluted 1: 100 in phosphate-buffered saline containing 0.05% (v/v) Tween 20 (PBS-T), biotinylated goat anti-human 19G (Amersham International PLC, Amersham, UK), streptavidin (Amersham International PLC, Amersham, UK), and biotinylated alkaline phosphatase (Boehringer-Mannhelm Blochemicals, Mannheim, Germany). Finally, 100 pi of p-nitrophenyl phosphate (Sigma, St Louis, MO, USA) at 1 mg/ml in 50 mM sodium carbonate buffer, pH 9.8, was added. The absorbance was read continuously at 405 rim (kinetic ELISA) using a computerized ELISA reader (Vmax®, Molecular Devices, CA, USA). All incubations were performed with continuous shaking and the plates were washed three times with PBS-T between each incubation step. Each serum sample was analyzed in duplicates, and in each immunoplate positive and negative standards were included. The reading of the optical density (mOD per min) for each sample was related to the positive standard on each immunoplate, and the data are presented as relative titres of antibody. The coefficient of variation of the positive and negative standards were 5.7 :t 2.9 and 8.1 ± 3.5 (M+SD), respectively.

EXAMPLE 5

Analysis of serum peosinogen 1 (PQ1)

The assay was based on a non-competitive sandwich technique using a horseradish peroxidase (HRP)-labelled antibody specific for PG1 to detect PG1 bound to a stationary antibody. The latter antibody was immobilized on a microtitre plate and had affinity for a different antigenic site in PG1 than the enzyme-labelled antibody.

Assay of serum pepsinogen 1 (PG1) was carried out using Gastroset PG1 (Gastroset PG1 Cat. No. 67882, Orion Diagnostica, Espoo, Finland) according to the manufacturer's instructions. Aliquots of 20 µL of standards, control and serum sample were added in duplicate into microtitre wells precoated with stationary pepsinogen 1 antibody. Assay buffer (100 µL) was added and the microtitre wells incubated for 30 minutes, washed twice, and then incubated for another 30 minutes with horseradish peroxidase-labelled PGI (HRP-PGI) antibody diluted 1:100 with assay buffer. The wells were washed four times and then incubated with substrate solution for 30 minutes; the reaction was stopped and colour development measured in an ELISA-reader.

Statistical Analysis

Results are presented as median and interquartile range (25th-75th percentiles). Proportions (percentages) are given with 95 percent confidence interval (CI), when considered relevant. Wilcoxon's signed-rank test was used to evaluate differences between pairs of patient groups. The level of significance was $p<0.05$.

EXAMPLE 6

Assays of sera from the study groups N, DU, AG and PA

Enzyme-linked immunosorbent assay

Sera of four groups of subjects that had their diagnosis at endoscopy and histological examination of biopsies were analyzed for H,K-ATPase antibodies (FIG. 1 A), H. pylori antibodies (FIG. 1 B), and pepsinogen (PGI) (FIG, 1 C). The product f-[relative titre of H. pylori antibodies x PG1] is presented in FIG. 1 D. The groups included subjects with a normal gastric mucosa.^ n=50), duodenal ulcer (DU, n=53), mild to severe corpus predominant atrophic gastritis (AG, n=46), and pernicious anaemia (PA, n=50). The results are presented as box plots with median and interquartile range (25th-75th percentiles, Hspread). Values outside the inner and outer fences are plotted with asterisks and open circles, respectively, and the upper fences are defined as the interquartile range + 1.5 Hspread and + 3 Hspread, respectively (SYSTAT® manual). Values outside the y-axis are presented in parenthesis. Significance is denoted by ** ($p<0.01$) and *** ($p<0.001$); n.s. not significant.

H.K-ATPase antibodies - In the study group constituting subjects with normal gastric mucosa (N), the median H,K-ATPase antibody titre was 2.0 (range'0.7-9.0; FIG. 1A). Corresponding values in the DU, AG, and PA groups were 9.3 (range 1.6-82.7), 3.8 (range 0.8-137) and 32.3 (range 1.4-128), respectively ($p< 0.001$ vs. normals).

Helicobacter pylori antibodies - The median titre of H. pylori antibodies in group N was 1.0 (range 1.04,5; Fig. 1 B). Corresponding values in the DU, AG, and PA groups were 47.5 (range 4.1-136), 18.0 (range 1.0-53.0) and 3.5 (range 1.3-27.2), respectively ($p<0.001$ vs. normals).

Serum pepsinogen 1 - The median value of serum PG1 in group N was 66,3 P9 per L (range 213-163; Fig. 1 C). Corresponding values in. the DU, AG, and PA groups were 149 pg per L (range 47.2-500), 31.8 pg per L (range 2.6-127), and 4.4 pg per L (range 0-61.0), respectively ($p<0.001$ vs. normals).

The factor f = (titre of H. pylori antibodies x serum DeDsinogen 1). Although the patient groups differed significantly using the three types of serologic parameters, there was an overlap. The titres of H. pylori antibodies and the PG1 concentrations were high in the DU group and low in the PA group. Therefore, in an attempt to distinguish better between the different groups, the products (f) of the analytical results from the ELISAs of H. pylori antibodies times the PG1 were evaluated (Fig. 1 D). All groups were significantly different from the others ($p<0.01$ or $p<0.001$, Fig. 1 D). The median value of f in group N was 77.5 (range 24.0-344). In the DU group it was 8105 (range

19340303; p< 0.001 vs. normals), in AG 500 (range 5.0-3901; p<0.001 vs. normals), and in PA 12.0 (range 0-915; p<0.01 vs. normals).

EXAMPLE 7

Evaluation scheme (flow chart) for serologic diagnosis

The status of the gastric mucosa was determined by means of histopathological examination of biopsy sections. This made possible a comparison of the gold standard with the results of serologic analyses. A diagnostic evaluation scheme was developed (FIG, 2). The discriminating levels of this scheme were optimized using the results of the serologic analyses (after omitting serologic outliers) in the study groups N, DU, AG and PA. The SYSTAT® software was used to achieve a maximal resolution between the patient groups . The "analytes" were H,K-ATPase antibodies (HK), H. pylori antibodies (HP), s-pepsinogen 1 (PGI), and f= (titre of H. Pylori antibodies x serum pepsinogen i) (HP*PG1). This scheme sorted the individual sera into the serologic groups 1-4 and their subgroups.

The evaluation scheme was applied to the analytical results from each individual serum which was sorted and grouped accordingly. Table 1 shows the serologic grouping of the four study groups: normal (N), duodenal ulcer (DU), corpus predominant gastritis without pernicious anaemia (AG) and pernicious anaemia (PA). The scheme for serologic diagnosis given in FIG. 2 was applied to the serological data.

The grouping resulted in the serologic subgroups 1A - 4C. The discriminating levels are indicated for the selection pathway in each subgroup and the median value of each group is indicated in parenthesis. The distribution of the study groups, sex and age in each serologic group are presented. In group 1 A, 84% (42/50) of the subjects in group N were obtained. Some sera from subjects in group N were serological outliers (e.g., in groups 113-1 D, 3A and 4A). In the groups 2A-2D, 91 % (48/53) of subjects with DU were obtained. In groups 3A and 3B, 57% (26/46) of those with AG and 18% (9/50) of those diagnosed with PA were obtained. In groups 4A4C, 72% (36/50) of those with PA and 22% (10/46) of those with AG were obtained. This overlap between the AG and PA group is not surprising since they represent the same disease, but on a different point of the time scale. Furthermore, an individual overlap was indicated by the 14% of the subjects with AG found in group 2. Otherwise the overlaps appeared small. The overall sensitivity to detect gastritis was 98% (146/149) (95% CI 94-100%) and the specificity 84% (42/50) (95% CI 71-93%).

Table 1

Serological Group	0.041667	1B	1C	1D	0.083333333	2B
Selection Pathway						
HK	<10(2.0)	<10(2.6)	<10(1.0)	>10(24.0)	< 10 (4.2)	<10 (7.1)
HP	<5 (1.0)	<5 (2.0)	<5 (1.0)	<5 (2.0)	>5 (46.0)	>5 (1 *O'M*
PG1	30-150(70)	<30(17)	>150(163)	>30(43)	-136	-122
HP*PG1	.-83	-20	-163	-129	>2000(6755)	<2000(1216)
PG1	-	-	-	-		>90
HP*PG1						-
Clinical Groups						
n=199	45	7	1	4	30	2
Normal (50)	42	3	1	2	0	0
DU (53)	2	0	0	0	23	2
AG (46)	0	1	0	1	7	0
PA (50)	1	3	0	1	0	0
Sex						
M 123 (62%)	35 (77.8%)	4 (57.1 %)	1	2(50%)	21(70%)	1 (50%)
F 76 (38%)	10 (22.2%)	3 (42.9%)		2(50%)	9(30%)	1 (50%)
Age						
median 67	e6	60	71	69	59	43
range 20-83	37-80	38-75_		61-78	20-79	38148

Table 1 (cont.)							
Serological Group	2C	2D	2n	.3 B	0.166667	413	4C
Selection Pathway							
HK	>10 (25.0)	>10 (10.3)	<10 (2.0)	>10 (42.0)	<10 (2.3)	>10 (67.0)	>10 (39.0)
HP	>5 (53.5)	>5 (11.4)	>5 (18.5)	>5 (17.0)	>5 (7.0)	>5 (6.0)	-,5(2.0)
PG1	-150	-148	-36	-23	-9	-4	<30 (8)
HP*PG1	>2000 (9232)	<2000 (1602)	<2000 (673)	<2000 (425)	<2000 (79)	<2000 (28)	<150 (8)
PG1	-	>90	<90	<90	<90	<90	-
HP*PG1	-	-	>150	>150	<150	<150	
Clinical Groups							
n=l 99	22	2	24	15	5	11	31
Normal (50)	0	0	1	0	1	0	0
DU (53)	21	2	1	2	0	0	0
A G (4 6)	1	0	17	9	-2	3	5
PA (50)	0	0	5	4	2	8	26
Sex							
M 123 (62%)	18 (81.8%)	1 (50%)	12(50%)	6(40%)	3(60%)	5 (45.5%)	14 (45.2%)
F 76 (38%)	4 (18.2%)	1 (50%)	12(50%)	9 (60%)	2 (40%)	6 (54.5%)	17 (54.8%)
Age							
median 67	53	58	70	68	72	73	70
range 20-83	23-79	48-68	40-83	34-82	54-82	54-82	44-81

FIGS. 3A-3D present the distribution profiles of the four major histomorphologically diagnosed groups identified by serology: group 1A, comprising 94% N, 4% DU and 2% PA; group 2 (A-D) comprising 86% DU and 14% AG; group 3 (A-B) comprising 66% AG, 23% PA, 8% DU, and 3% N; and group 4(A-C) comprising 77% PA, 21 % AG, and 2% N. Together groups 3 and 4 comprised 95% of subjects in groups AG and PA (42% and 53%, respectively), 2% N, and 3% DU. A smaller group represented by groups 1 B-D in Table 1, comprised twelve subjects of whom 50% belonged to group N, but serologically they were outliers. The remaining six subjects in these subgroups had either AG (17%) or PA (33

EXAMPLE 8

Serologic diagnosis in a sample of the general population

A sample of 483 subjects (age 37 to 85 years) randomly selected from a general population in Sweden was previously examined with WD with biopsy and blood sampling. Fifty percent (243/483) had a normal gastric mucosa and the remaining had gastritis (Borch, K, et al., Dig Dis Sci, 2000, 45: 1322-29). In the present study sera from this population were analysed and grouped according to the scheme in FIG. 2 and the outcome compared with that of the histomorphological diagnosis. Table 2 shows the serologic grouping of the general population sample. The scheme for serologic diagnosis given in FIG. 2 was applied to the serologic data. The grouping resulted in the serologic subgroups 1 A - 4C. The discriminating levels are indicated for the selection pathway in each subgroup and the median value of each group is indicated in parenthesis. The distributions of the histomorphologically diagnosed groups, sex, and age in each serologic subgroup are presented. The morphological diagnose were normal (0), non-atrophic antrum predominant gastritis (1), atrophic antrum predominant gastritis (2), non-atrophic pangastritis. (3), atrophic pangastritis (4), non-atrophic corpus predominant gastritis (5), and atrophic corpus predominant gastritis (6).

Table 2

Serological Group	0.041666667	1B	Lc	-1 D	0.08333333	2B
Selection Pathway						
HK	<10 (1.8)	<10(1.5)	<10(1.6)	>10(17.8)	< 10	< 10 (2.3)
HP	<5 (0.9)	<5 (1.1)	<5 (1.2)	<5 (1.1)	>5 (30.3)	>5 (11.2)
PG1	30-150(63)	<30 (22)	> 150 (227)	>30 (64)	-96	-107
HP*PG1	-56	-22	-248	-60	>2000(2691)< 2000(1189)	
PG[	-	-	-	-		>90
HP*PG1	-					-
Histomorphological Diagnosis						
n=483	225	18	7	25	38	22
0 (normal) 243 (50.3%)	196	16	5	20	-	2
1 (ant) 20 (4.1%)	5	-	1	1	3	1
2 (ant d) 87 (18.1%)	12		1	-	14	5
3(pan)74 (15.3%)	5		-	1	14	13
4 (pan a) 14 (2.9%)	0		-	-	6	-
- 5 (corp) 13 (2.7%)	7	1	-	2		1
6 (corp a) 32 (6.6%)	0	1	-	1	1	
Sex						
M 261 (54%)	127 (56.4%)	7 (38.9%)	6 (85.7%)	11 (44.0%)	23(60.5%)	16(72.2%)
F 222 (46%)	98 (43.6%)	11 (61.1%)	1 (14.3%)	14 (56.0%)	15(39.5%)	6(27.3%)
Age						
median (65)	56	52	66	62	65	63
range (37-85)	37-81	38-74	47-85	37-78	44-81	41-74

Table 2 (cont.)

Serologic Group	2C	2D	0.1251	3B	10.1666667	4B	4C
Selection Pathway							
HK	>10 (30.3)	>10 (69.4)	<10 (2.0)	>10 (31.1)	<10 (2.2)	>10 (70.5)	>10 68.5)
HP	>5 (28.4)	>5 (12.2)	>5 (16.4)	>5 (18.8)	>5 (7.4)	>5 (7.3)	<5 (1.5)
PG1	-141	-112	-61	-49	-8	-9	<30 (8)
HP*PG1	>2000 (4140).	<2000 (1318)	<2000 (953)	<2000 (805)	<2000 (62)	<2000 (61)	<150 (18)
PG1		>90	<90	<90	<90	<90	-
HP*PG1	-	-	>'I 50	>150	<150	<150	
Histomorphological Diagnosis							
n=483	10	3	95	28	2	4	6
0 (normal) 243 (50.3%)		-	4	-	-	-	-
1 (ant) 20 (4.1%)	1	-	8				
2 (ant a) 87 (18.1%)	3	2	39	11			
3 (pan) 74 (15.3%)	5	1	26	9			
4 (pan a) 14 (2.9%)	1	-	5	2			
5 (corp) 13 (2.7%)	-		1	-	-	-	1
6 (corp a) 32 (6.6%)			12	6	2	4	5
Sex							
M 261 (54%)	7 (70%)	3 (100%)	44 (46.3%)	12 (43%)	2 (100%)	2 (50%)	1 (17%)
F 222 (46%)	3(30%)	--	51 (53.7%)	16 (57%)	-	2 (50%)	5 (83%)
Age							
Fnmedian (65)	63	67	63	68	75	76	72
range (37-85)	44-73	58-72	38-80	43-80	47-82	69-80	50-8707]

In group 1A, 87% (196/225) of the subjects exhibited serologic values indicating a normal gastric mucosa. Seven percent (16/243) of the subjects with normal gastric mucosa had low levels of PG1 and were obtained in group 1 B, while 8% (20/243) were obtained in group 1 D due to increased levels of H,K-ATPase antibodies. Six percent (29/483) of the population sample diagnosed as having gastritis of any type were obtained in group 1A.

In groups 2A-21D, 15% (73/483) were obtained by means of the serologic grouping procedure (Table 2). In groups 2A-2D only two subjects had normal mucosa. The dominating morphological diagnoses in groups 2A-21D were antrum predominant gastritis with atrophy (24 subjects) and pangastritis without atrophy (33 subjects). Subjects in groups 3A-313 exhibited a positive serology for H. pylori. In group 3A 20% (95/483) of the population were obtained and the dominating morphological diagnoses were antrum predominant gastritis with atrophy (39 subjects), pangastritis without atrophy (26 subjects), and pangastritis with atrophy or corpus predominant atrophic gastritis (17 subjects). In groups 4A-4C the PGI values were generally low which indicated a more pronounced corpus atrophy. Only twelve subjects were found in these groups and they had corpus predominant gastritis with atrophy. The overall sensitivity to detect gastritis serologically in the population was 88% (211/240) (95% CI 83-92%) and the specificity 81% (196/243) (95% CI 75-85%).

The distribution profile according to serology in the four major groups of the sample of the general population is presented in FIGS. 4A-4D The distribution profiles of the histomorphologically diagnosed groups in the serologic groups 1A 2(A-D), 3(A- and 4(A-C) are presented. The morphological diagnoses were normal (0) , nonatrophic antrum predominant gastritis (1), atrophic antrum predominant gastritis (2), non-atrophic pangastritis (3), atrophic pangastritis (4), non-atrophic corpus predominant gastritis (5), and atrophic corpus predominant gastritis (6).

In group 1A, the morphologically assessed normals comprised 87% (196/225). In group 2, antrum gastritis with atrophy, comprising 33% (24173), and pangastritis, comprising 45% (33/73), were the dominating groups. In group 3 (A-B), antrum gastritis with atrophy, 40% (50/123), pangastritis, 28% (35/123), and atrophic corpus gastritis, 15% (18/123) were the dominating groups. In group 4 (A-C), atrophic corpus gastritis comprised 92% (11/12) and non-atrophic corpus gastritis the remaining 8% (1/12).

The dominating morphological diagnoses in groups 3 plus 4 were atrophic antrum predominant gastritis, 35% (50/145), pangastritis, 24% (35/145), and corpus predominant atrophic gastritis, 20% (29/145). These three subgroups comprised 79% (114/145) of the subjects in groups 3 plus 4. The prevalence of atrophic corpus predominant gastritis was 7% (321483) in the population, 91 % (29/32) of these were obtained in groups 3 plus 4.

Although the preferred embodiment of the method and kit of the invention has been described above in some detail, it should be appreciated that a variety of embodiments

will be readily apparent to one skilled in the art. The description of the method and kit of this invention is not intended to be limiting to this invention, but is merely illustrative of the preferred embodiment.

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CLAIMS

1. A method for diagnosing possible presence of gastritis in a human by evaluating a blood sample, comprising assaying the blood sample for the presence of antibodies specific for H,K-ATPase, antibodies specific for Helicobacter pylori, and the concentration of pepsinogen I, whereby the presence of H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I concentration are compared between themselves and in relation to the respective values of H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I concentration of a normal population, in a software related system, wherein altered levels in the sample is indicative of gastritis.

2. A method according to claim 1, wherein an altered level detection leads to the issuance of a remittance for further investigation with regard to gastritis.

3. A method according to claim 1, wherein the step of determining the levels of said indicators comprising performing immunoassays for detecting the indicators.

4. A method according to claim 1 or claim 2, wherein the group of indicators includes an additional indicator comprising the level of pepsinogen I multiplied by the level of Helicobacter pylori antibodies, and wherein the level of this additional indicator is compared to a standard.

5. A method according to one or more of claims 1-3, wherein the levels of H,K-ATPase antibodies and Helicobacter pylori antibodies, which are significantly higher than the levels in a normal population is indicative of gastritis.

6. A method according to one or more of claims 1-3, wherein a lowered level of pepsinogen I concentration is indicative of corpus atrophy.

7. A method according to one or more of claims 1-3, wherein an increased level of pepsinogen I concentration is indicative of a corpus gastritis, optionally without any autoimmunity involved.

8. A method according to one or more of claims 1-3, wherein the level of H,K-ATPase antibodies differing from that of the normal population is indicative of an autoimmune corpus atrophy.

9. A method according to one or more of claims 1-3, wherein the level of Helicobacter pylori antibodies differing from that of the normal population is indicative of antrum, or pangastritis.

10. A method according to one or more of claims 1-3, wherein increased levels of Helicobacter pylori antibodies, and normal to lowered concentrations of pepsinogen I is indicative of atrophy.

11. A method according to one or more of claims 1-3, wherein very low concentrations of pepsinogen I in combination with increased levels of H,K-ATPase antibodies is indicative of corpus atrophy.

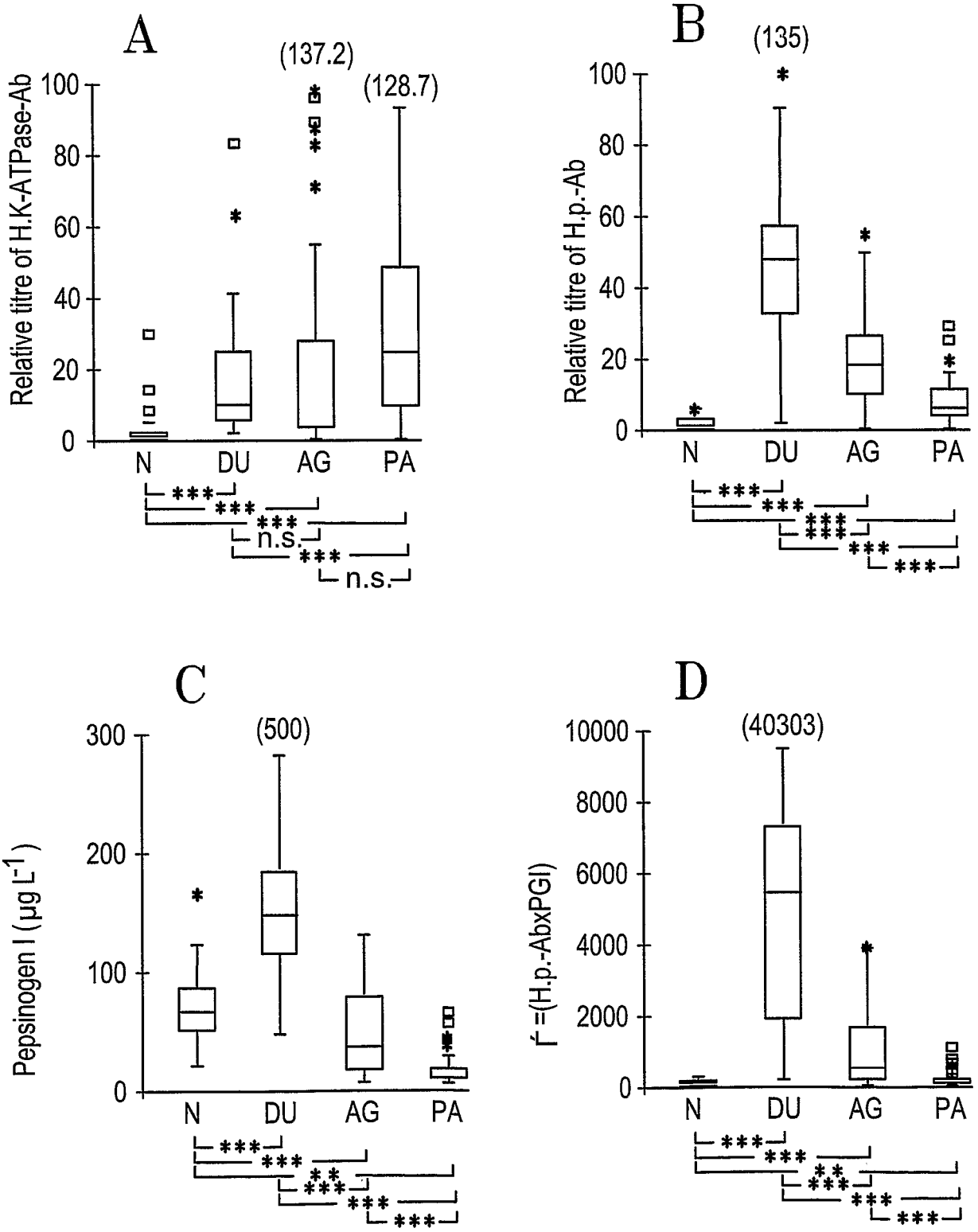
12. A kit for screening for gastritis comprising reagents suitable for detecting H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I concentration.

13. A kit according to claim 11, wherein the reagents comprise pepsinogen I antibodies, H,K-ATPase and Helicobacter pylori proteins or peptides thereof.

14. A kit according to claim 11, wherein the reagents comprise pepsinogen I, H,K-ATPase and Helicobacter pylori antigens immobilized on a solid support.

15. A kit according to claim 13, wherein it further comprises labelled anti-human antibodies.

16. A kit according to claim 11, wherein the reagents are provided in amounts sufficient to perform substantially equal numbers of assays to detect H,K-ATPase antibodies, Helicobacter pylori antibodies, and pepsinogen I concentration.


A
(137.2)
(128.7)
Relative titre of H.K-ATPase-Ab
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80
60
40
20
0
N
DU
AG
PA
***
***
***
n.s.
***
n.s.
B
(135)
Relative titre of H.p.-Ab
100
80
60
40
20
0
N
DU
AG
PA
***
***
***
***
***
***
C
(500)
Pepsinogen I (µg L-1)
300
200
100
0
N
DU
AG
PA
***
***
**
***
***
***
D
(40303)
Γ´=(H.p.-AbxPGI)
10000
8000
6000
4000
2000
0
N
DU
AG
PA
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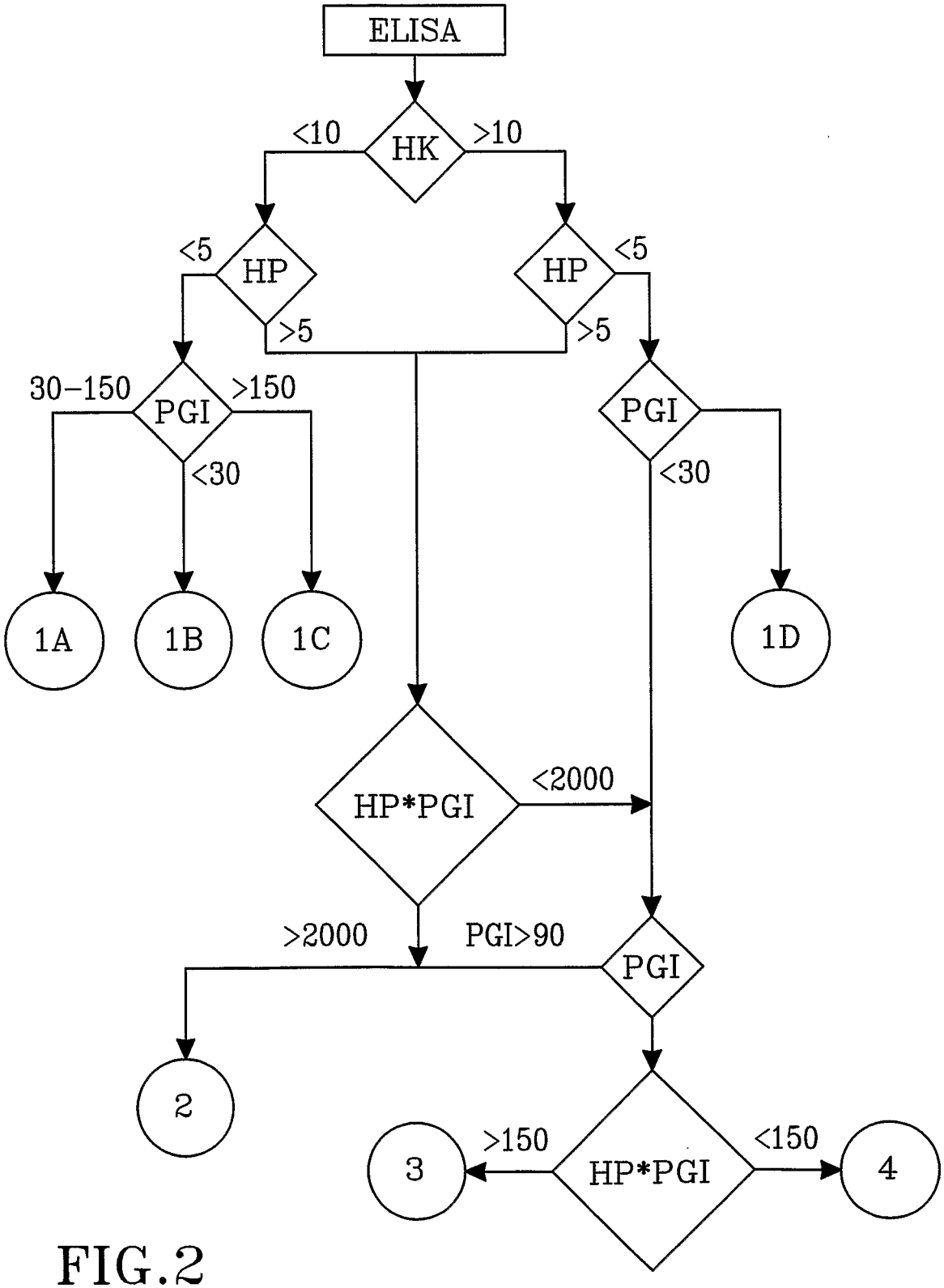
FIG. 1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HP
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>5
PGI
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>150
<30
PGI
<30
1A
1B
1C
1D
HP*PGI
<2000
>2000
PGI>90
PGI
2
3
>150
HP*PGI
<150
4

FIG.2

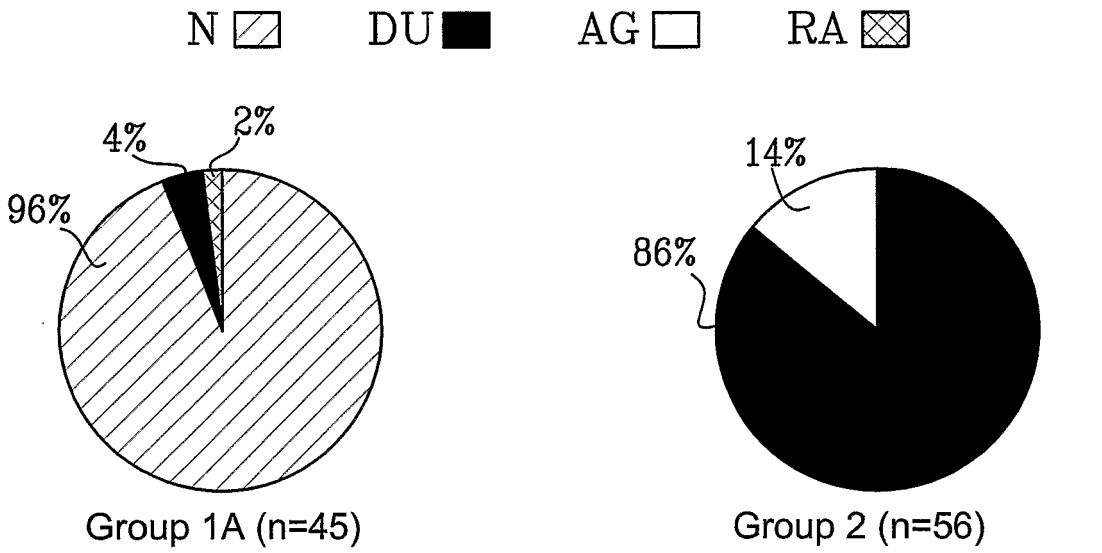


FIG.3A

FIG.3B

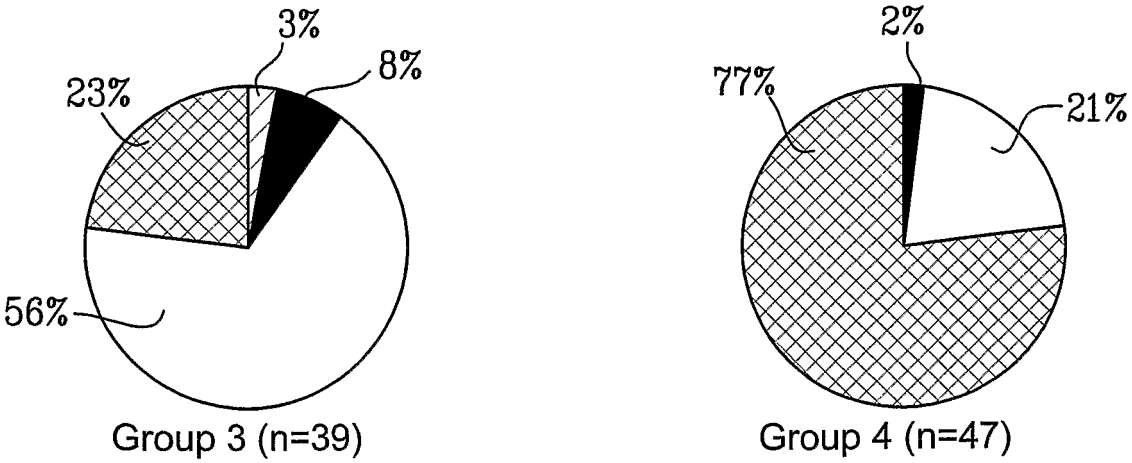
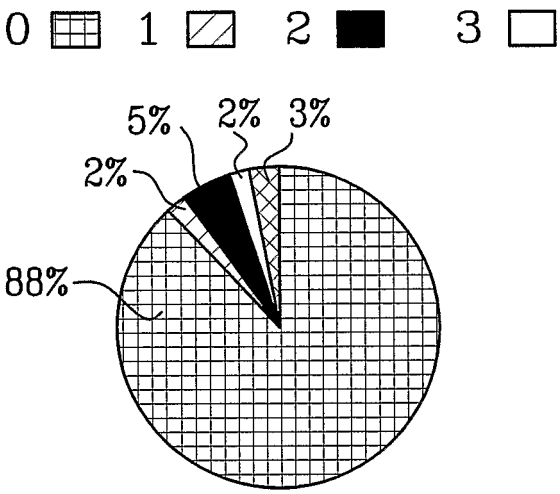


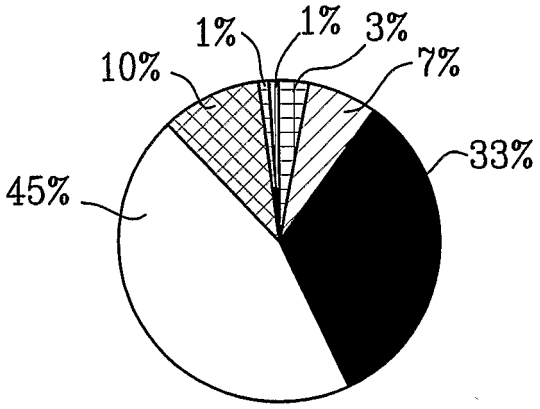
FIG.3C

FIG.3D



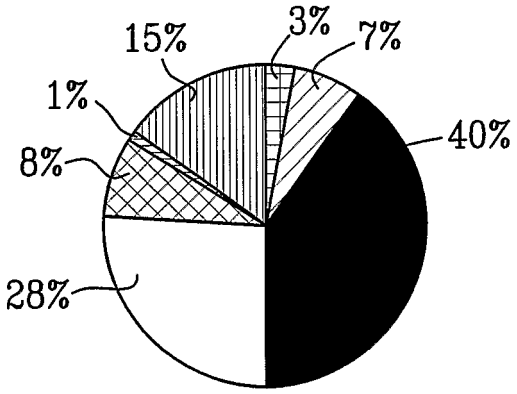
Group 1A (n=225)

FIG.4A



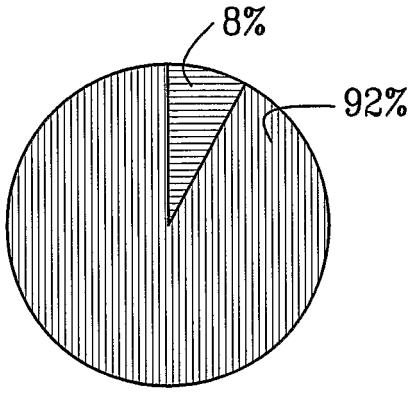
Group 2 (n=73)

FIG.4B



Group 3 (n=123)

FIG.4C



Group 4 (n=12)

FIG.4D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 03/00469

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/564, G01N 33/68, G01N 33/573
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	European Journal of Gastroenterology & Hepatology, Volume 10, 1998, Anders Lindgren et al: "Serum antibodies to H+,K+-ATPase, serum pepsinogen A and Helicobacter pylori in relation to gastric mucosa morpholoyg in patients with low or low-normal concentrations of serum cobalamins", pages 583-588 ---	1-3,5-7,9, 10,12-16
X	Scandinavian Journal of Gastroenterology, Volume 37, 2002, M. Ito et al: "Role of Anti-Parietal Cell Antibody in Helicobacter pylori-associated Atrophic Gastritis: Evaluation in a Country of High Prevalence of Atrophic Gastritis", pages 287-293 ---	1-3,5-7,9, 10,12-16

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 July 2003	09 -07- 2003
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer CARL-OLOF GUSTAFSSON/BS Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 03/00469

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	European Journal of Gastroenterology & Hepatology, Volume 14, 2002, Masanori Ito et al: "Serological comparison of serum pepsinogen and antiparietal cell antibody levels between Japanese and German patients", pages 123-127 --	1-3,5-7,9, 10,12-16
A	WO 0067035 A1 (LOCUS GENEX OY), 9 November 2000 (09.11.00) -- --------	1,12

INTERNATIONAL SEARCH REPORT

Int nal application No.
PCT/SE03/00469

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-11
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Claims 1-11 relate to methods of treatment of the human or animal body by surgery or by therapy/diagnostic methods practised on the human or animal body/Rule. 39.1.(iv)). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

Internauonal application No.
PCT/SE 03/00469

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0067035 A1	09/11/00	AU 4299800 A	17/11/00
		CN 1355888 T	26/06/02
		EP 1173770 A	23/01/02
		FI 990992 A	31/10/00
		JP 2002543433 T	17/12/02

专利名称(译)	胃炎的筛查方法		
公开(公告)号	EP1488238A1	公开(公告)日	2004-12-22
申请号	EP2003745056	申请日	2003-03-21
[标]申请(专利权)人(译)	艾特罗弗斯公司		
申请(专利权)人(译)	ATROPHUS AB		
当前申请(专利权)人(译)	ATROPHUS AB		
[标]发明人	MARDH SVEN MARDH ERIK		
发明人	MARDH, SVEN MARDH, ERIK		
IPC分类号	G01N33/573 G01N33/53 G01N33/569 G01N33/68 G01N33/564		
CPC分类号	G01N33/6893 G01N2800/062		
优先权	0200974 2002-03-27 SE		
其他公开文献	EP1488238B1		
外部链接	Espacenet		

摘要(译)

本发明涉及一种通过评估血样来诊断人胃炎可能存在的方法，包括测定血样中是否存在对H，K-ATP酶，幽门螺杆菌特异性抗体特异性的抗体的浓度，以及胃蛋白酶原I，其中H，K-ATP酶抗体，幽门螺杆菌抗体和胃蛋白酶原I浓度的存在在它们自身之间以及相对于H，K-ATP酶抗体，幽门螺杆菌抗体和胃蛋白酶原浓度的相应值进行比较。在软件相关系统中，正常人群，其中样本中改变的水平指示胃炎，并且优选地，改变的水平检测导致发出汇款以进一步研究胃炎。