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SUPERINTENDENCIA DE INDUSTRIA Y COMER

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Solicitud de Patente de Invención titulada: "Un método mediante una prueba de aliento para la detección de microorganismos patógenos"

Solicitante: Germán Antonio Campuzano Maya

MEMORIAL DE COMPLEMENTACIÓN

ÁLVARO CORREA ORDÓÑEZ, mayor de edad y vecino de Bogotá D.C., identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad en referencia, presento respetuosamente los siguientes documentos que complementan mi respuesta a la oposición que presenté el pasado 23 de julio de 2008:

- 3 artículos de revista de Estados Unidos de América, Inglaterra y Corea; y
- Tesis (Valutazione) en donde referencia internacionalmente la prueba de aliento, solicitada en patente de la referencia como una nueva prueba con sensibilidad y especificidad de 100%, en la literatura médica mundial.

Atentamente,

ÁLVARO CORREA ORDÓÑEZ

C.C.No.79.143.366 de Usaquén

T.P.20.281

Anexos:

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Imaging and Advanced Technology

Michael B. Wallace, Section Editor

Breath Tests for Gastrointestinal Disease: The Real Deal or Just a Lot of Hot Air?

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Although endoscopy provides an accurate means by which to identify structural or histologic abnormalities of the gastrointestinal (GI) tract, it is invasive, expensive, and carries a small risk of complications. As such, there remains a need for simple, noninvasive, less expensive tests that can provide similar or supplemental information to endoscopy. Several breath tests have been developed to fill this void. This review discusses the rationale and evidence available to support the most commonly utilized breath tests in clinical practice.

Urea Breath Test for *Helicobacter pylori* Infection

The urea breath test (UBT) is the most accurate and best validated of the currently available breath tests. The UBT is an accurate noninvasive method of identifying active *Helicobacter pylori* (*H pylori*) infection before and after antibiotic therapy. This test relies upon the oral administration of urea labeled with ^{13}C or ^{14}C to identify urease, an enzyme produced in large quantities by *H pylori*. Urease cleaves the labeled urea to ammonia and labeled bicarbonate, which is subsequently converted to labeled carbon dioxide that is then measured in exhaled breath (Figure 1).

The earliest versions of the UBT utilized small amounts (1–10 μCi ; 1 μCi is less than typical daily background radiation exposure) of the radioactive isotope, ^{14}C , to label urea. The exhaled $^{14}\text{CO}_2$ can be measured using a scintillation counter. Sensitivity and specificity of the ^{14}C -UBT for *H pylori* has consistently been reported to be $>90\%$.¹

An alternative UBT utilizing the nonradioactive isotope, ^{13}C , is the most widely used version of this test. Given the absence of radiation exposure, the ^{13}C -UBT is most appropriate for children and pregnant women. A recent multicenter study that evaluated the ^{13}C -UBT in children reported a sensitivity of 96%–98% and specificity of 96%–99% for *H pylori* compared with biopsy-based testing.² The greatest limitations of the ^{13}C -UBT are the high cost of the substrate and the isotope ratio mass spectrometer needed for breath analysis. Newer analyzers offering greater convenience and lower cost include a tabletop instrument utilizing molecular correlation spectrometry³ and an infrared spectrometer.⁴ A recent study reported that a simplified

^{13}C -UBT protocol that utilized a smaller dose of ^{13}C -urea (50 mg), no test meal, and a single, 10-minute breath collection yielded 100% accuracy for the diagnosis of *H pylori*.⁵ Similar results have been reported with simplified ^{14}C -UBT protocols.⁶

The sensitivity of the UBT can be reduced by the recent use of proton pump inhibitors (PPIs), bismuth-containing compounds, or antibiotics. Most experts suggest that PPIs should be withheld for 1–2 weeks and bismuth and antibiotics should be withheld for ≥ 4 weeks before the UBT. Standard doses of histamine-2 receptor antagonists do not affect the sensitivity of the UBT.⁷

Carbohydrate Breath Tests

A number of carbohydrate breath tests have been utilized to assess orocecal transit, carbohydrate malabsorption, or identify patients with small intestinal bacterial overgrowth (SIBO; Table 1). This breath testing modality relies on the ability of intestinal bacteria to metabolize various carbohydrate substrates producing hydrogen and/or methane and leading to the release of measurable levels of these gases in exhaled air from the lungs (Figure 2).

Before testing, it is important to instruct patients to avoid fiber and unfermentable carbohydrates such as pasta or bread on the evening before their test.⁸ False-positive results can occur in patients with poor oral hygiene. This can be minimized by rinsing with an antibacterial mouthwash before ingestion of test substrate. Smoking can lead to false-positive results and physical exertion can lead to hyperventilation and false-negative results; both activities should be avoided for >2 hours before testing.

Given the need for specialized equipment, dedicated space, and technical support, hydrogen breath testing tends to be confined to larger centers. Because up to one third of individuals have microflora that do not produce hydrogen, it is mandatory for breath analyzers to measure hydrogen and methane. It is also useful for analyzers to have a “self-correction” feature that accounts for carbon dioxide in the

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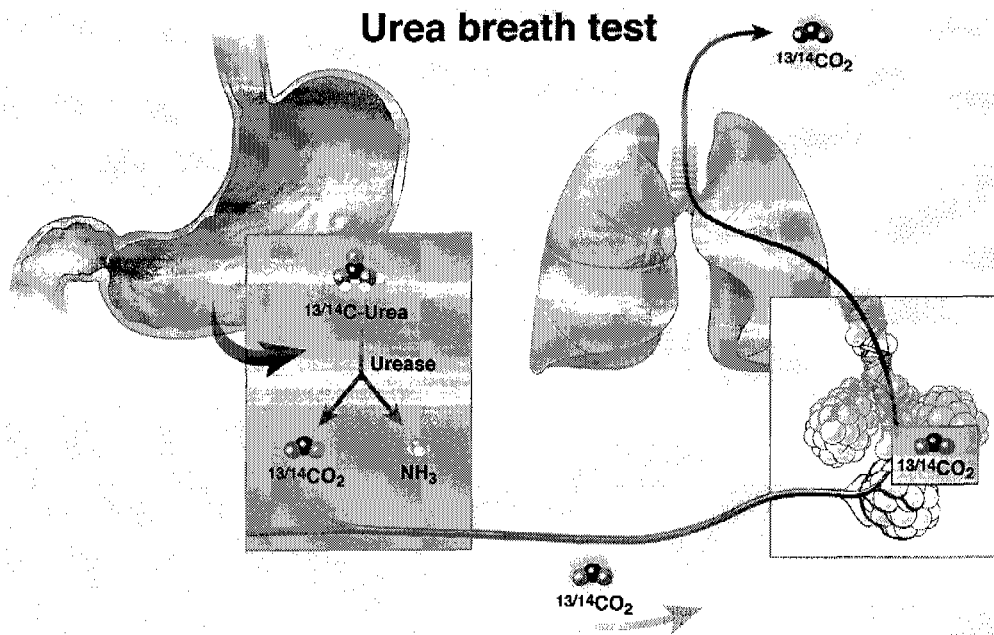


Figure 1. Schematic for urea breath testing.

breath sample. This helps to minimize errors in the event that a breath sample was imperfectly collected.

Several portable devices capable of use in virtually any office setting have been developed offering comparable accuracy to the traditional laboratory gas analyzer equipment. A novel commercially available portable breath analyzer (E-Z-EM H₂) has been compared with the standard laboratory breath analyzer in SIBO and found to have a high correlation of measured gas values at all time points (*r*-value range 0.87–0.97; *P* > .001).⁹ An earlier portable breath analyzer (Micro H₂) was also shown to closely correlate with the standard laboratory model in the diagnosis of

lactose malabsorption [overall agreement of 91% (95% confidence interval {CI}, 78%–97%) and yielded an identical diagnosis in 100% (95% CI, 92%–100%) of cases].¹⁰

Breath Tests for Carbohydrate Malabsorption

Hydrogen breath testing can be useful in the assessment of carbohydrate malabsorption. Lactose is a disaccharide metabolized by the brush border enzyme lactase to glucose and galactose. Reduction or absence of this enzyme leads to excessive exposure of the colon to lactose, where fermentation can result in symptoms such as bloating, excessive flatus, cramping, and loose stools in a subset of

Table 1. Commonly Utilized Carbohydrate Breath Tests

Indication	Substrate	Recommended Challenge Dose	Positive Findings
Suspected carbohydrate malabsorption	Lactose	25 grams	Rise in H ₂ or CH ₄ > 20 ppm
	Fructose	25 grams	Rise in H ₂ or CH ₄ > 20 ppm Or Successive incremental increase >5 ppm in 3 consecutive samples
Suspected small intestinal bacterial overgrowth	Lactulose	10 grams	Baseline H ₂ or CH ₄ >20 ppm Or A double peak in H ₂ or CH ₄ levels Or Early rise in H ₂ or CH ₄ >20 ppm Or Incremental increase in H ₂ or CH ₄ >10 ppm in consecutive samples
	glucose	50-75 grams	Baseline H ₂ or CH ₄ >20 ppm Or Rise in H ₂ or CH ₄ by 12-15 ppm

H₂, hydrogen gas; CH₄, methane gas; ppm, parts per million

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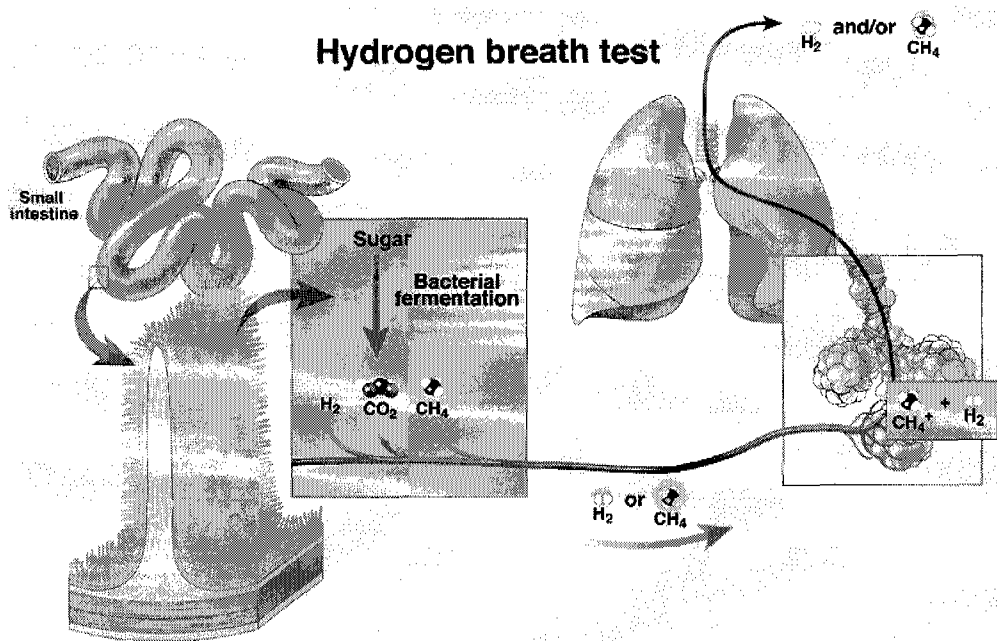


Figure 2. Schematic for carbohydrate breath testing.

affected persons.¹¹ Breath testing for lactose intolerance has been used by gastroenterologists for many years. Most centers give 25 g of lactose and collect breath samples over 2–4 hours. A positive test is typically defined as a rise in breath hydrogen or methane excretion of >20 parts per million (ppm).

Fructose is a monosaccharide used as a commercial sweetener and is also found in a number of fruits. Another important source of fructose is sucrose, a disaccharide composed of fructose and glucose. Fructose malabsorption has increasingly been identified as a potential cause of GI symptoms.^{12,13} A recent study attempted to identify the proper dosage of fructose for hydrogen breath testing.¹⁴ In this study, 20 healthy volunteers received 15–50 g of fructose in a double-blinded fashion. Based on hydrogen and methane measurements and a symptom questionnaire, a 25-g dose was found to most reliably identify patients with fructose malabsorption. The authors defined a positive test as a rise in breath hydrogen or methane excretion of >20 ppm or a rise of >5 ppm on consecutive breath samples as a positive test.

Breath Tests for SIBO

There has been a recent surge of interest in SIBO related to its possible clinical and physiologic overlap with irritable bowel syndrome (IBS).^{15,16} SIBO has traditionally been defined as >10⁵ cfu/mL on quantitative culture of a small intestinal aspirate. Unfortunately, pitfalls including technical difficulty, frequent contamination by oral flora, high cost, and the ability to sample only from the proximal small bowel make quantitative culture an imperfect gold standard for SIBO. In clinical practice, lactulose or glucose hydrogen breath testing is often used as a means of identi-

fying patients with SIBO. Each test has advantages and disadvantages.

Under normal circumstances, lactulose is not absorbed by the small intestine. Once lactulose reaches the colon, it is fermented by bacteria to short-chain fatty acids as well as hydrogen and/or methane gas. Based on this physiology, the lactulose breath test was originally developed as a surrogate measure of orocecal transit time. Because bacteria anywhere in the GI tract can ferment lactulose, this test has also been suggested to identify patients with SIBO.

One issue that makes the literature difficult to interpret is the lack of standardization of the lactulose breath test protocol. Most centers use 10 g of lactulose and a 2- to 4-hour breath sampling period. A variety of findings have been used to define a positive test including a fasting breath hydrogen level of >20 ppm, the presence of a “double peak” (the first peak representing lactulose metabolism by small intestinal bacteria and the second peak corresponding to lactulose reaching the cecum), an early rise in breath hydrogen concentration of >20 ppm, or >10 ppm on consecutive samples.^{8,17}

Unfortunately, the classic double peak is rarely seen in clinical practice. More commonly, a single peak in breath hydrogen and/or methane is found, which can result from fermentation occurring in the small bowel and colon as would be expected in the setting of SIBO or simply colonic fermentation as would be the case in an individual without SIBO. The later scenario holds particular relevance to patients with IBS and diarrhea who tend to have accelerated ileocecal transit.¹⁸ Acknowledging that there is currently no gold standard for the diagnosis of SIBO, the sensitivity and specificity of the lactulose breath test has been reported to

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be 68% and 44%, respectively, relative to quantitative small bowel culture.¹⁹ A recent study in IBS patients reported that 35% and 45% of IBS patients and controls had a positive lactulose hydrogen breath test, whereas only 4% of patients and controls had SIBO by jejunal aspiration for quantitative culture. Interestingly, 43% of IBS patients versus 12% of controls had bacterial counts of $>5 \times 10^3/\text{mL}$ on jejunal culture, raising numerous questions about differences in microflora and fermentation between those with and without IBS.²⁰

The other substrate used to evaluate for SIBO is glucose, which is avidly absorbed in the proximal small intestine. Most centers administer 50–75 g of glucose and collect breath samples for 2–3 hours. A positive test is defined as a baseline breath hydrogen or methane excretion of >20 ppm and/or a rise of >12 – 15 ppm after glucose ingestion. Because glucose typically does not reach the distal small bowel, the glucose breath test may theoretically be less sensitive than the lactulose breath test, although the literature is not clear on this issue. On the other hand, the glucose breath test is likely more specific than the lactulose breath test.¹⁹

Breath tests using D-xylose, ¹³C-xylose, and cholyglycine have been described in the literature,⁸ but have yielded conflicting results and are not widely available. Thus, these tests do not appear to offer clear advantages over the more conventional tests.

In summary, breath tests have become an integral part of clinical gastroenterology. The UBT is a validated means by which to identify *H pylori* infection before or after antibiotic therapy. A variety of breath tests can aid in the identification of carbohydrate maldigestion and malabsorption. However, the presence of an abnormal carbohydrate breath test does not prove cause and effect with GI symptoms. Lactulose and glucose breath tests are being used to identify patients with SIBO. Unfortunately, the sensitivity and specificity of these tests for SIBO is inadequate for routine clinical use. At present, it is not clear that a positive test predicts clinical outcomes after treatment for SIBO. Clearly, further work to improve upon the current generation of breath tests is warranted.

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Helicobacter pylori 감염 유무와 정도 반영에 대한 C-14와 C-13 요소호기검사 정량치 비교

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임석태 · 김동욱 · 정환정 · 손명희

Comparison of the Quantitative Values of C-14 and C-13 UBT to Reflect the Presence and Degree of Ongoing *Helicobacter pylori* Infection

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Purpose: A urea breath test (UBT) using C-14 or C-13 has been developed for identifying *Helicobacter* (*H.*) *pylori* infection on the basis of urease production with release of labeled CO₂. We investigated if the C-14 and C-13 UBT have the difference to reflect the presence and degree of *H. pylori* infection detected by gastro-duodenoscopic biopsies (GBx) in the same patients. **Materials and methods:** Thirty eight patients (M:F=28:10, age 53.4±13.0 yrs) with upper gastrointestinal symptoms such as indigestion, gastric fullness or pain consecutively underwent C-14 UBT, GBx and C-13 UBT within one week before medications. For the C-14 UBT, a single breath sample was collected at 10 minutes after ingestion of C-14 urea (37 KBq) capsule and counting was done in a liquid scintillation counter for 1 minute, and the results were classified as positive (≥200 dpm), intermediate (50~199 dpm) or negative (<50 dpm). For the C-13 UBT, the results were classified as positive (≥2.5‰) or negative (<2.5‰). The results of GBx with Giemsa stain were graded 0 (normal) to 4 (diffuse) according to the distribution of *H. pylori* by the Wyatt method. We compared C-14 UBT and C-13 UBT results with GBx grade as a gold standard. **Results:** The prevalence of *H. pylori* infection by GBx with Giemsa stain was 25/38 (65.8%). In the assessment of the presence of *H. pylori* infection, the C-14 UBT global performance yielded sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of 92.0%, 92.3%, 95.8%, 91.7% and 92.1%, respectively. However, the C-13 UBT had sensitivity, specificity, PPV, NPV and accuracy of 96.0%, 84.6%, 92.3%, 91.7% and 92.1%, respectively. The more significant correlation in C-14 than C-13 UBT ($r=0.948$ vs $r=0.819$, $p<0.001$) was found between the value of UBT and the grade of distribution of *H. pylori* infection. **Conclusion:** We conclude that the diagnostic performance between C-14 and C-13 UBT to detect *H. pylori* infection is not significantly different, but the value of C-14 UBT more significantly reflects the degree of bacterial distribution. (Nucl Med Mol Imaging 2008;42(3):229-234)

Key Words: urea breath test, C-14, C-13, *Helicobacter pylori*

서 론

Helicobacter pylori (이하 *H. pylori*)는 1982년 Warren과 Marshall에 의해 만성 위염 환자의 위 생검 조직에서 처음으

로 동정된 이후 이에 대한 연구가 활발하게 진행되어 위염, 위궤양, 십이지장궤양 등의 양성 위십이지장 질환 뿐만 아니라 위암의 위험인자로 알려져 있다.¹⁻⁵⁾ 따라서 *H. pylori* 감염과 계균 여부에 대한 정확한 진단이 소화성 궤양 환자의 치료에 필수적 요소가 되고 있다.

H. pylori 감염의 진단은 침습적인 방법으로 내시경을 통한 생검조직을 이용하여 균배양을 하거나 urease 활성도를 이용하여 간접적으로 *H. pylori* 감염 여부를 측정하는 rapid urease test (이하 CLO 검사)와 Warthin-Starry silver 또는 modified Giemsa 염색 후 현미경검사 등이 있으며 비침습적인 방법으로는 C-14 또는 C-13 요소호기검사(이하UBT),

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혈청학적 검사, 대변내 항원검사 등이 있다.⁶⁻⁹⁾ UBT는 침습적인 내시경검사가 필요 없으며 간편하고 저렴하며 비교적 정확하게 *H. pylori* 감염 여부를 진단하는데 유용한 것으로 알려져 있다. 최근 들어 *H. pylori*에 의한 염증이 진행시 만성 위축성 위염을 일으키고 나아가서는 위암을 일으킨다는 학설이 대두되면서 UBT가 *H. pylori* 감염의 경중도를 정량적으로 예측할 수 있는지에 대한 연구가 다양하게 진행되고 있다.¹⁰⁻¹³⁾ 하지만 아직까지는 동일한 환자에서 시행된 C-14와 C-13 UBT 측정치와 조직생검 소견에서 관찰되는 *H. pylori* 감염 정도를 비교한 연구보고는 찾아 보기가 어렵다.

저자들은 *H. pylori* 감염 여부를 진단하고자 1주일 이내에 C-14와 C-13 UBT, 위십이지장내시경을 통한 조직생검 검사를 모두 시행하여 C-14와 C-13 UBT의 진단성능을 알아보고 각각의 정량적 측정치가 조직생검에 의한 *H. pylori* 분포 정도와 유의한 상관관계가 있는지를 알아보려고 하였다.

대상 및 방법

1. 대상

소화불량, 위팽만감, 상복부동통과 상부위장관 증상이 있어 1주일 이내에 C-14와 C-13 UBT, 위십이지장내시경을 통한 조직생검 검사를 모두 시행한 38명(남:녀=28:10, 나이: 53.4±13.0세)의 환자를 대상으로 하였다. 대상환자 중에서 최근 1개월내에 bismuth제제나 항생제를 복용하였거나 2주 이내에 H₂ 길항제나 proton pump 억제제를 복용한 환자, 위장관절제술을 받은 환자, 상부위장관 출혈이 있는 환자는 대상에서 제외하였다.

2. 방법

1) 위십이지장 내시경 및 조직생검 검사

대상환자는 내시경 검사전에 8시간 이상 금식하였고, 전 처치로 검사 15분전에 hyoscine-N-butylbromide (Buscopan[®]) 20 mg과 midazolam (Dormicom[®]) 1 mg을 근육주사하고, 2% lidocaine HCl 10 ml로 인후부의 국소마취를 시행한 후 수세소독된 내시경(GIF-Q200, Olympus, U.S.A)으로 검사를 받았다. 궤양 및 미란 부위와 장상피화 부위를 피하고 유문륜으로부터 2 cm 이내의 위유문부로부터 2편의 조직을 채취하여 병리조직검사를 시행하였다. 조직의 양을 같게 하기 위하여 한 종류의 검사만을 이용하여 가능한 최대의 조직을 채취하였다. 내시경검사로 얻은 조직은 Giemsa 염색을 실시하였으며 Wyatt법^{14,15)}을 이용하여 균의 분포에 따라 0에서 4까지 등급을 정하였으며, 등급 0은 *H. pylori* 감염에 대하여 음성으로, 등급 1-4는 양성으로 판정하였다. 등급 0은 조직

절편 내에서 *H. pylori*를 발견할 수 없는 경우, 등급 1은 한두 개 정도의 점막에서 인접한 균을 발견할 수 있는 경우, 등급 2는 위소와들과 위전막 표면의 50% 이내에서 균을 관찰할 수 있는 경우, 등급 3은 50% 이상에서 균을 발견할 수 있는 경우, 등급 4는 조직절편 내에서 *H. pylori*의 치밀한 집락이 있는 경우로 정의하였다.

2) C-14 요소호기검사

위십이지장내시경검사 후 약 1시간 뒤에 상품화된 PYtest[®] 키트(BALLARD, Utah, U.S.A)를 이용하여 37 KBq (1 μ Ci)의 C-14으로 표지된 urea가 들어 있는 캡슐을 20 ml의 증류수와 함께 경구 복용시키고 3분 후에 20 mL의 증류수를 추가로 마시게 한 후 10분에 최대로 흡기후 5초간 숨을 참게 한 뒤에 알루미늄으로 된 풍선을 이용하여 호기속에 있는 이산화탄소를 채집하였다. 채집 후 2.5 ml의 포집용액이 들어 있는 유리로 된 용기에 공기펌프를 이용하여 C-14가 있는 이산화탄소를 포집한 후 섬광용액 10 ml를 첨가하여 고루 섞이게 하고 전용 베타카운터(microCOUNT LITE, BALLAD, U.S.A)로 측정하였다. 1999년 미국핵의학학회에서 제시한 기준에 따라 측정치가 50 dpm이하인 경우에는 음성, 200 dpm 이상인 경우에는 양성으로 판정하였고, 50에서 200 dpm 사이로 측정된 경우에는 1시간 뒤에 재측정하여 50 dpm이하인 경우에는 음성, 200 dpm 이상인 경우에는 양성으로 판정하였고, 지속적으로 50에서 200 dpm 사이에 해당되는 경우에 미분류로 판정하였다.¹⁶⁾

3) C-13 요소호기검사

위십이지장내시경과 C-14 UBT 시행 후 1주일 이내에 4시간 이상 금식한 환자를 대상으로 UBiT[®] 키트(Korea Otsuka Pharmaceutical Co. Ltd., Seoul, Korea)를 이용하여 C-13 urea 100 mg이 들어 있는 캡슐을 복용하기 전의 호기와 복용 후 20분이 지난 호기를 포집하여 전용 적외분광분석 장치(UBiT-IR300, Korea Otsuka Pharmaceutical Co. Ltd., Seoul, Korea)로 ¹³CO₂ 및 ¹³CO₂/¹²CO₂ 비를 측정하고 그 변화량($\Delta^{13}C$)을 산출해서 2.5‰ 이상을 양성, 2.5‰ 미만을 음성으로 판정하였다.

4) 병리조직검사에 대한 C-14와 C-13 요소호기검사의 진단율 비교

병리조직검사 결과를 금과옥조로 하여 C-14와 C-13 UBT의 *H. pylori*에 감염 여부 진단에 대한 예민도와 특이도 양성예측도, 음성예측도, 정확도를 비교분석 하였다.

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Table 1. Comparison of Diagnostic Performance between C-14 and C-13 Urea Breath Test with Gastroduodenoscopic Biopsies

C-14 UBT*	GBx†			C-13 UBT	GBx		
	Positive	Negative	Total		Positive	Negative	Total
Positive	23	1	24	Positive	24	2	26
Intermediate							
Negative	2	12	14	Negative	1	11	12
Total	25	13	38		25	13	38

*UBT, urea breath test

†GBx, the result of gastroduodenoscopic biopsy with giemsa stain

Table 2. Comparison of Concordance between C-14 and C-13 Urea Breath Test

	C-14 UBT*			Total
	Positive	Intermediate	Negative	
C-13 UBT				
Positive	23		3	26
Negative	1		11	12
Total	24		14	38

*UBT, urea breath test

5) 병리조직 검사 등급에 따른 C-14 와 C-13 요소호기검사의 정량적 수치 상관관계 비교

Wyatt법에 의한 조직의 균의 분포에 따른 등급(0-4)과 C-14 와 C-13 요소호기검사에 의하여 측정된 정량치와의 상관관계를 SPSS 통계프로그램의 Kruskal-Wallis 비모수 검정법과 스피어맨 순위상관계수를 구하여 분석하였고 p값이 0.05 미만인 경우를 유의하다고 판정하였다.

결 과

1. 병리조직검사에 대한 C-14 와 C-13 요소호기검사의 진단을 비교

대상환자 38명에서 Giemsa 염색을 시행한 병리조직검사에서 양성을 보인 환자는 25명(65.8%)이었고, 음성을 보인 환자는 13명(34.2%)이었다. 조직검사에서 양성을 보인 25명 중에서 C-14 UBT에서 양성을 보인 환자는 23명이었고, 나머지 2명에서는 음성을 보였다. 반면에 C-13 UBT에서는 24명에서 양성을 보였고, 음성은 1명이었다(Table 1). 조직검사 결과를 최적 기준(gold standard)로 하였을 경우, *H. pylori* 감염 진단에 있어 C-14 UBT의 민감도, 특이도, 양성예측도, 음성예측도, 정확도는 92.0%, 92.3%, 95.8%, 85.7%, 92.1%를 보였다. C-13 UBT는 민감도, 특이도, 양성예측도, 음성예측도, 정확도가 96.0%, 84.6%, 92.3%, 91.7%, 92.1%를 보였다. C-14 와 C-13 UBT는 23명에서 모두 양성을 보였고 11명에서는 모두 음성을 보여 *H. pylori* 감염을 진단하는데 두 검사는 89.5%(34/38)에서 일치하였고 10.5%(4/38)에서

서로 다른 결과를 보였다(Table 2).

2. 병리조직 검사 등급에 따른 C-14 와 C-13 요소호기검사의 정량적 수치 상관관계 비교

Wyatt법에 의한 병리조직 검사에 대한 등급을 정하였을 때 음성으로 판명되는 등급 0는 13명이었고 양성을 보인 25명 중에서 등급 1은 5명(20%), 등급 2는 7명(28%), 등급 3은 9명(36%), 등급 4는 4명(16%)으로 등급 3를 보인 환자가 가장 많았다. 조직학적 등급에 대하여 C-14 UBT 측정치는 유의한 상관관계($r=0.948$, $p<0.001$)를 보였고, C-13 UBT 측정치도 유의한 상관관계($r=0.819$, $p<0.001$)를 보였으나 C-14 요소호기검사가 좀 더 높은 상관관계가 있었다(Fig. 1).

고 찰

UBT가 *H. pylori* 감염 여부를 진단하는데 있어서 유용하다 보고는 많이 있다.¹⁷⁻²⁰⁾ 하지만 UBT의 측정된 정량치가 *H. pylori* 감염의 정도나 분포를 반영할 수 있는지에 대한 연구보고는 적은 편이다. 이 연구는 *H. pylori* 감염 정도를 진단하는데 있어서 C-14 과 C-13 UBT의 진단성능을 동일환자에서 반복 시행함으로써 정확하게 비교하였고, 두 UBT의 측정된 정량치와 Wyatt법에 의한 조직학적 검사결과를 최적 기준으로 삼아 균의 분포 등급을 비교하였다.

조직검사 결과를 최적 기준으로 하였을 때 *H. pylori* 감염에 대한 진단성능 비교시 C-13 UBT가 C-14 UBT에 비하여 유의한 차이는 아니지만 민감도는 96.0%로 약간 높으나 특

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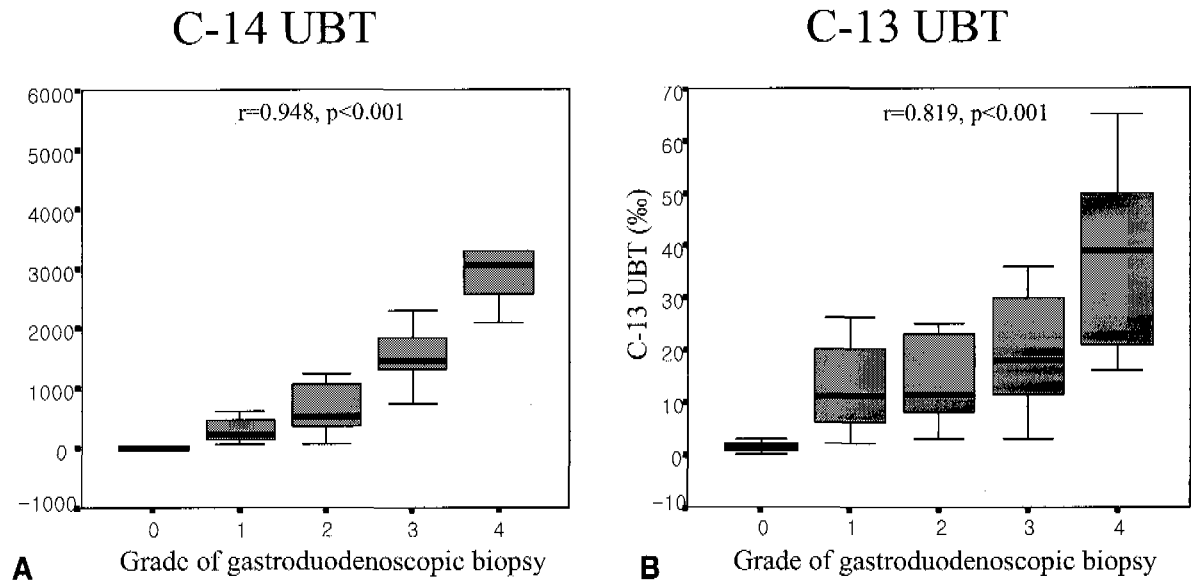


Figure 1. Quantitative values of C-14 and C-13 urea breath tests according to pathologic grading. A significant correlation was found between C-14 UBT (A) and C-13 UBT (B) values and the grade of distribution of *H. pylori* infection. However, the correlation coefficient of the C-14 UBT was more excellent than that of the C-13 UBT.

이도는 84.6%로 낮은 결과를 보였다. 하지만 두 검사에서 양성예측도의 차이는 큰 차이가 없으며 음성예측도와 정확도는 동일하였다. 즉 *H. pylori* 감염을 진단하는데 있어 두 검사의 정확도는 92.1%를 보이고 있어 차이는 없을 것으로 생각된다. 이전에 보고²⁰⁻²²⁾된 연구에서의 UBT의 민감도와 특이도가 97-100%, 95-100%인데 비하여 저자들의 연구에서 다소 낮은 값을 보인 것은 대상 환자군의 차이에 의한 것으로 생각된다. 이는 C-14 UBT의 민감도는 92.0%로 저자들이 이전에 보고한 150명의 유사한 환자군을 대상으로 한 연구¹⁵⁾에서와 비슷한 결과를 보인 것으로 추측 할 수가 있을 것이다. 또한 이 연구의 복제가 동일한 환자군을 대상으로 하여 C-14과 C-13 UBT의 진단 성능을 비교하는 것이기 때문에 타 연구와 진단 성능 비교는 의의가 적을 것이라고 생각된다. 결론적으로 *H. pylori* 감염을 진단하는데 C-14과 C-13 UBT는 유의한 차이가 없으므로 진료 여건에 따라 선택하여 사용하여도 무방할 것이라고 생각된다.

최근 들어 *H. pylori*에 의한 염증이 진행 시 만성 위축성 위염을 일으키고 나아가서는 위암을 일으킨다는 학설이 대두되면서 *H. pylori* 감염의 경중도를 간편하게 예측할 수 있는 UBT가 널리 이용되고 있다.^{10,12,13)} 특히 간단하면서도 빠르고 정확하면서도 비침습적으로 *H. pylori*를 진단하는 방법에 대한 연구 보고가 지속적으로 이루어지고 있다. 이 연구는 C-14 또는 C-13 UBT에서 측정된 정량치로 *H. pylori* 감염 정도를 예측하는데 도움이 되는지와 도움이 된다면 차이

가 있는지를 비교하고자 하였다. 지금까지 동일 환자군을 대상으로 하여 이러한 연구 보고는 없어 비교적 38명의 적은 수의 환자군을 대상으로 하였지만 임상적 의의는 클 것이라고 생각한다. Figure 1에서 보여주듯이 C-14 UBT가 *H. pylori* 감염 등급을 구분하는데 C-13 UBT에 비하여 좀 더 우수한 것으로 여겨진다. 따라서 *H. pylori* 감염을 진단하거나 진단 후 군박멸요법으로 치료하고 나서 재발 유무를 검사하는데 C-14 또는 C-13 UBT는 모두 유용하게 이용될 수 있으며, *H. pylori* 감염 및 분포 정도를 평가하는 목적으로는 C-13 UBT에 비하여 C-14 UBT가 좀 더 효과적이라고 여겨진다.

UBT는 체내에서 *H. pylori*에 의해 생성된 urease에 의해 C-13 또는 C-14 표지 탄소가 있는 HCO_3^- 가 혈액 내로 흡수된 후 이산화탄소로 변화되어 호기로 배출되는 양을 측정하는 방법으로 정량적 측정이 가능하며 임상적으로 군박멸요법 후 추적 관찰 시에 침습적인 내시경 검사에 의한 조직 생검이 필요한 CLO검사를 대체하여 사용되고 있는 간편한 방법이다. C-13 UBT는 비방사성 동위원소를 사용함으로써 방사능 피폭이 없다는 장점이 있으나 비용이 많이 들고 결과를 보는데 시간이 걸리며 복잡하고 비싼 질량분석 장비가 필요하다. 검사식(test meal)과 차가운 urea 투여가 필요하다는 단점이 있어 이용이 제한되어 있다. 반면에 C-14 UBT는 구강내의 urease에 의한 위양성을 방지하기 위한 캡슐제재로 되어 있어 검사 전에 양치질이나 구강세척 등의 전처치가 필요 없으며, C-13 UBT와는 달리 일회용 호기 채취 만으로도

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되는 간편한 방법이다. 또한 대부분의 대형병원에 액체섬광 계수기가 구비되어 있어 가격도 저렴하면서 검사 당일에 결과를 볼 수 있어 미국을 비롯한 서구 선진국에서 보편적으로 사용되고 있다. Stubbs 등²³⁾의 연구에 의하면 37 KBq 정도의 C-14 urea 투여시 받는 선량당량은 11시간 정도 자연방사선으로 받는양과 비슷한 정도이고, Leide-Svegborn 등²⁴⁾의 연구에 의하면 110 KBq의 C-14으로 표지된 요소를 사용한 경우에 성인의 유효선량이 2.1 uSv이고 55 KBq의 C-14를 이용한 소아의 경우에는 0.9-2.5 uSv로 극히 적은 피폭이어서 최소 7세까지의 어린이에서도 안심하고 사용할 수 있다고 하였다.

이번 연구의 제한점은 첫째, *H. pylori* 감염에 최적 기준으로 삼은 내시경 검사가 다양한 부분에서 위조직 생검을 시행하지 못하고 위유분부 2cm 이내에서 조직 검사가 이루어져서 정확하게 *H. pylori* 감염 정도를 평가하지 못한 점이다. 하지만 현실적으로 위 전체에서 조직 검사를 시행하는 것은 비효율적이고 *H. pylori*가 가장 흔한 침범 부위가 위유분부이고 유문부와 기저부의 일치율이 96.4%로 높다는 보고가 있어 유문부에서의 결과를 최적 기준으로 하여도 큰 문제는 없을 것으로 사료된다. 둘째, C-14 과 C-13 UBT 가 같은 날에 시행하지 못한 점이다. 같은 날에 두 검사를 시행하였을 경우에 서로 다른 검사에 영향을 미칠 가능성이 높아 정량치에 대한 신뢰성이 줄어 들 가능성이 있어 같은 날에 두 검사를 시행하지 않았다. 하지만 두 검사 사이의 간격을 1주일 이내로 하였고 또한 균박멸요법 시행 전으로 하여 두 검사 사이에 미칠 수 있는 요인을 최소화 하였다.

결론적으로 *H. pylori* 감염을 진단하거나 진단 후 균박멸요법으로 치료하고 나서 재발 유무를 검사하는데 C-14 또는 C-13 UBT는 모두 유용하게 이용될 수 있으며, *H. pylori* 감염 및 분포 정도를 평가하는 목적으로는 C-13 UBT에 비하여 C-14 UBT정량치를 사용하는 것이 좀 더 유용할 것으로 여겨진다.

요 약

목적: 최근 들어 *H. pylori*에 의한 염증이 진행되면 만성 위축성위염을 일으키고 나아가서는 위암을 일으킨다는 학설이 대두되면서 UBT의 이용이 증가되고 있다. 저자들은 *H. pylori* 감염과 분포 정도를 평가하는데 C-14 와 C-13 UBT의 진단능력과 유용성에 차이가 있는지를 알아보았다. **대상 및 방법:** 소화불량, 위궤판감, 상복부동통 등의 상부위장관 증상이 있어 위십이지장내시경을 시행 받은 환자 중 균박멸요법을 시행 전에 조직검사, C-14 및 C-13 UBT를 모두 시

행 받은 38명(남:녀=28:10, 나이: 53.4±13.0세)을 대상으로 하였다. 조직생검 결과를 최적 기준으로 하여 C-14 와 C-13 UBT의 진단능력을 비교하였고 Wyatt법에 의한 조직검사의 등급(0-4)과 C-14 및 C-13 UBT의 정량적 측정치와의 상관관계를 비교하였다. **결과:** 조직생검에서 25명(65.8%)에서 *H. pylori* 감염에 대한 양성을 보였다. *H. pylori* 감염 진단하는데 C-14 UBT는 민감도 92.0%, 특이도 92.3%, 양성예측도 95.8%, 음성예측도 91.7%, 정확도는 92.1% 이었으나, C-13 UBT는 민감도 96.0%, 특이도 84.6%, 양성예측도 92.3%, 음성예측도 91.7%, 정확도는 92.1% 이었다. 조직검사 등급에 따른 C-14 UBT 측정치와의 상관성이 C-13 UBT 측정치에 비하여 우수하였다(각각 $r=0.948$ vs $r=0.818$, $p<0.001$). **결론:** *H. pylori* 감염을 진단하는데 C-14 와 C-13 UBT는 유의한 차이는 없으나 C-14 UBT 측정치가 *H. pylori* 감염 정도를 평가하는데 좀 더 유용할 것으로 생각된다.

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Diagnosis of *Helicobacter pylori*

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Keywords

Culture, urease test, histology, urea breath test, serology, stool antigen test, PCR.

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Abstract

The different invasive and noninvasive diagnostic tests for *Helicobacter pylori* have been applied mainly in emerging countries. Molecular methods have been developed, especially a test for detection of *H. pylori* and its clarithromycin resistance directly from stools. The long-term effects of eradication on histologic lesions have been studied in a meta-analysis and the prognostic value of post-treatment in gastric mucosa-associated lymphoid tissue lymphoma has been assessed. An operating link for gastritis assessment (the OLGA staging) has also been published. Attempts to simplify the urea breath test protocol have been made, and new stool antigen tests have been proposed and compared to those previously available.

During this past year, diagnosis of *Helicobacter pylori* has been the subject of several reviews [1,2] and numerous original articles mostly coming from emerging countries that reported the use of the different methods in their setting have been published.

Invasive Tests

Despite the numerous attempts to replace invasive tests by noninvasive tests, the need to observe the gastric mucosal lesions and to obtain *H. pylori* for further studies remains.

Endoscopy

Previous attempts to diagnose *H. pylori* gastritis by standard endoscopy were not successful. In another study the authors looked at antrum nodularity as a test for *H. pylori* gastritis in children and adolescents and found a good sensitivity, while a regular arrangement of collecting venules pattern, as indicative of normal gastric mucosa, had a good specificity [3]. In addition, the new method of magnifying endoscopy, e.g. narrow-band imaging, appears to be good predictor of gastric histologic diagnosis [4].

Culture

Culture is reputed to be difficult for this fastidious bacterium. Given the ability of *H. pylori* to colonize the gastric mucosa, Takahashi et al. tried to find an eventual growth factor from murine gastric mucosal cells [5]. They could purify a substance enhancing *H. pylori* growth by 100-fold as assessed by colony count. The substance was identified by mass

spectrometry to be L-lactic acid, and a L-lactic acid-dependent growth of *H. pylori* was observed. This important discovery deserves confirmation.

Interestingly, the role of *H. pylori* culture from duodenal biopsies was raised by Pietroiusti et al. in the case of idiopathic duodenal ulcer. They could find that almost half of the 6.9% of duodenal ulcer patients *H. pylori*-negative in the stomach had *H. pylori* colonization in the duodenum and the main risk factor for such cases was previous antibiotic consumption [6].

Culture from stools is rarely successful even if the bacteria are present, possibly because they are in a non-culturable stage. Nevertheless, *H. pylori* culture was compared to HpSA on stools from children in Iran. The colonies obtained were tested with anti-*H. pylori* monoclonal antibodies and polymerase chain reaction (PCR) for *H. pylori ureA* gene in addition to standard tests. They claimed the growth of coccoidal *H. pylori* when *H. pylori* density in gastric biopsies was important. A cholestyramine treatment of stools improved the recovery of viable *H. pylori* [7].

Another *Helicobacter*, *Helicobacter cinaedi*, can be found in humans in systemic infections. A study of 16,743 blood culture samples in 13 Tokyo hospitals was carried out during 6 months to know its prevalence. Six isolates of *H. cinaedi* were identified out of nine *Helicobacter* species (0.06%). Most of the patients had a chronic underlying disease, e.g. malignancy, renal failure, while no HIV-positive patient was found [8].

During this year, six articles have dealt with the culturability of *H. pylori* maintained in water. The methodology was similar, i.e. *H. pylori* strain(s) was (were) introduced in a laboratory water model, and the culturability was tested

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on agar. *H. pylori* was found to be culturable by Azevedo et al. up to 4 days, i.e. a much longer period than the six enterohepatic *Helicobacters* tested in parallel [9]. Other survival studies also found a positive culture after 4 or 5 days while viability decreased progressively during 14 days with a progressive transformation from spiral to coccoidal forms [10,11]. Konishi et al. reported a better survival of *H. pylori* after storage in groundwater and even in seawater than in nutrient broth [12]. In contrast, chlorinated water was detrimental for *H. pylori* only after 5 minutes (negative culture) at a concentration of 0.96 mg/L while viable bacteria could be detected by fluorescence in situ hybridization (FISH) during 3 hours [13]. These studies indicate that *H. pylori* can be considered as a waterborne pathogen during its viable stage. Finally, *H. pylori* selective media should be developed for marine samples because current media are not selective enough [14].

Culture is also an excellent method to detect enterohepatic *Helicobacter* species in mice to be used for biomedical research. A survey of 79 mice from 34 sources worldwide yielded 62 positive (78%) by culturing cecal content. They used a combination of CVA (cefoperazone, vancomycin, and amphotericin B) plates, TVP (trimethoprim, vancomycin, and polymyxin B) plates, filters onto trypticase soy agar plates with 5% sheep blood, and in-house-prepared selective medium plates. *Helicobacter hepaticus* was the predominant species but others could be found [15].

Molecular Methods

Different formats of PCR are still being proposed. Gill et al. used a *ureC*-based PCR with the isothermal digoxigenin (DIG)-labeling tHDA process. It allowed to detect the amplicons with a specific biotinylated DNA probe and gave excellent sensitivity and specificity compared to culture [16]. Chisholm and Owen used a multiplex PCR for *H. pylori* speciation and two real-time probe hybridization assays for clarithromycin and tetracycline susceptibility testing. It allowed them to improve their detection rate by 30% compared to culture [17]. Real-time PCR performances were also favorable in comparison to histology (sensitivity 98.2 vs. 87.7%) in a study by Tankovic et al. [18]. A nested PCR targeting *Hsp60* was found of interest for *H. pylori* diagnosis [19].

The value of PCR results is more problematic when this method is applied to other specimens such as dental plaque [20] or in the environment (water, sewage). A new quantitative PCR with SYBR green targeting the *vacA* gene was developed and found 100-fold more sensitive [11]. Then, 84% of their 24 sewage samples were positive.

An alternative to PCR is FISH detection. This is a rapid and reliable method for routine application on frozen biopsies [21,22].

Urease Test

The urease test remains the most rapid and cost-effective invasive method. A South Korean study evaluated the impact of atrophy and intestinal metaplasia on the sensitivity of the CLOtest. The positivity of CLOtest was found to be lower in atrophy with intestinal metaplasia than in atrophy without intestinal metaplasia (50 vs. 80% in the antrum) [23].

A new urease test namely HUITAI rapid urease test has been proposed. The result is read after 60 minutes but the median positive reaction time was 1 minute. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 98.2%, 99%, 99%, 97.9%, and 98.5%, respectively [24].

Histology

Shiotani et al. focused on antral nodularity in young adults. It was identified in two-thirds of *H. pylori*-positive subjects. Nodular gastritis was divided into diffuse or nondiffuse types. The diffuse type predominantly affected women [odds ratio (OR) 3.9 95% confidence interval (CI) 1.5–10]. The atrophy scores in the lesser curve of the antrum were significantly higher in the nondiffuse than in the diffuse group [25].

After successful *H. pylori* eradication and normalization of the endoscopic findings in gastric mucosa-associated lymphoid tissue lymphoma, minimal histologic residuals are still present at 12 months. Based on a follow up of 42 months of a series of 100 cases, 62% had stable minimal histologic residuals while progression was seen in 5% arguing for a wait-and-watch strategy [26].

The long-term effects of *H. pylori* eradication on gastric histology were also studied in a meta-analysis. A significant improvement of gastric atrophy was noted, whereas no improvement was shown for intestinal metaplasia, indicating that it could be a "point of no return" [27].

Another interesting information during this year was the publication of a tutorial by an international group of experts who defined an Operative Link for Gastritis Assessment: the OLGA staging for gastritis. This staging arranges the histologic phenotypes of gastritis along a scale of progressively increasing gastric cancer risk from the lowest (0) to the highest (IV) in order to give to practitioners an explicit message of value for clinical management [28].

Noninvasive Methods

Urea Breath Test

A trend in urea breath test (UBT) was to simplify the established protocol. UBT protocols were evaluated and

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compared to the European protocol in a study from Columbia, presenting also a tabular summary of the literature [29]. A simplified ^{13}C -UBT protocol with no test meal gave the best results at 100% for all performance parameters. A study from South Africa [30] compared the simpler and cheaper GC-MS to the standard IRMS and found a sensitivity and specificity of 92% and 93%, respectively. Another simplified ^{13}C -UBT showed a sensitivity and specificity of 90.2% and 93.3%, respectively [31]. A more economic ^{13}C -UBT with a maximum of 25 mg (UBT²⁵) used in Taiwanese children had a sensitivity and specificity of 88.9% and 95.0%, respectively, while accuracy was 98% in children > 7 years of age, and significantly higher than the 85% in younger children ($p = .009$) [32]. ^{13}C -UBT was evaluated in hospitalized patients with peptic ulcer bleeding and treated with proton-pump inhibitors in Spain [33]. Of the 131 patients, 86% had a first positive UBT while repeated UBT was positive in 15 of 18 (83%) negatives at first UBT.

An attempt to correlate delta over baseline (DOB) values with the outcome of eradication therapy was made in Italy [34] but found no significant difference in DOB values by treatment outcome or by the antibiotic resistance pattern to clarithromycin. The predictive value of ^{13}C -UBT in the choice of second- and third-line eradication therapy was explored in 134 duodenal ulcer patients with a failed first eradication therapy, finding a negative correlation between ^{13}C -UBT values and outcome both in the second- and the third-line treatments [35]. A meta-analysis of *H. pylori* recurrence after eradication identified ten valid studies from developed countries and seven from developing countries that used ^{13}C -UBT for follow up [36]. The calculated annual recurrence rates were 2.67% and 13.0%, respectively. With a longer follow up, the recurrence rate was 1.45% and 12.0%, respectively.

Stool Antigen Test

In stool samples from children, Schwarzer et al. [37] evaluated the new rapid office-based one-step monoclonal immunoassay (RAPID Hp StAR) and a monoclonal-based ELISA targeting the same antigen (Hp StAR). The HpStAR showed a pretreatment sensitivity and specificity of 98% and 100%, respectively, the corresponding data post-treatment being 100% and 96.2%. The rapid assay, however, had technical problems, interobserver variability, and a low performance.

In Korea [38], the rapid HpSA ImmunoCard Stat and the ELISA Premium platinum HpSA both from Meridian (Milan, Italy) were compared in children before treatment. Sensitivity of both the rapid assay and the ELISA was 94.6%, while specificity was 98.4% and 96.1%, respectively. After therapy, both assays had a sensitivity and specificity of

88.9% and 91.7%, respectively. A study from Mexico [39] also evaluated the rapid HpSA ImmunoCard Stat in children and found a sensitivity and specificity 96.3% and 95.1%, respectively. In contrast to these studies, a study from Iran [40] on children showed a sensitivity and specificity of only 54.8% and 79.4%, respectively, for the Equipar polyclonal HpSA ELISA. In Greek adults, a conventional ELISA (Novitec®, Hiss Diagnostics, Freiburg, Germany) showed after adjustment of the cut-off value a sensitivity and specificity of 82% and 94%, respectively. For the rapid assay (Stick *H. pylori*®, Operon S.A., Zaragoza, Spain) both parameters were only 78% [41].

Six stool antigen assays were compared for performance before and after eradication treatment [42]. The sensitivity and specificity were 87.3% and 83.3% for Immundiagnostik ELISA, 92.5% and 72.2% for HpSA EIA test, 95% and 66.6% for HpStAR EIA, 83.8% and 66.6% for *H. pylori* Letitest, 52.5% and 94.4% for ImmunoCard HpSA, and 78.8% and 55.5% for RAPID HpStAR, respectively. The best agreement between the ^{13}C -UBT and stool immunoassays was with HpStAR EIA (90%) and *H. pylori* Letitest (85%). A study from Italy [43] evaluated the rapid test ImmunoCard STAT HpSA and the Amplified IDEA Hp StAR after eradication therapy and found that the rapid Hp StAR had a sensitivity and specificity of 58.1% and 76.4%, respectively, while the corresponding data for the IDEA Hp StAR were 97.5% and 98.7%, respectively. In Malaysia, Bhewa et al. showed that the monoclonal IDEA Hp StAR had both a sensitivity and specificity of 97.8% [44].

A study from Turkey [45] investigated the quantitative correlation between the Premier Platinum HpSA and the severity of *H. pylori*-associated gastritis. After adjustment of the cut-off level, the assay had a sensitivity and specificity of 92% and 100%, respectively. A significant ($p < .05$) correlation was found between high values in the HpSA and high scores of corpus *H. pylori* load as well as to the severity of antrum and corpus inflammation. In Gambia [46], the HpSA was found to give less equivocal results in the youngest infants than ^{13}C -UBT. Agreement between the tests was poor in the young infants but increased with age, reaching 86% by 12 months of age.

PCR in Stools

An in-house PCR method [47] was evaluated in 74 patients, of whom 34 were on proton pump inhibitors and 20 on anti- H_2 , while the remaining were untreated. The authors concluded that for the patients on antisecretory therapy, the sensitivity of both rapid urease test (RUT) and histology is reduced and PCR is more sensitive in these patients.

In a study from South Africa [48] an in-house PCR was used to investigate the prevalence of *H. pylori*, finding a low rate of 50.6%.

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Another study [49] evaluated the ClariRes assay (Ingenctix, Vienna, Austria) for detection of *H. pylori* and for a simultaneous clarithromycin susceptibility testing. This novel biprobe real-time PCR showed 100% specificity, while sensitivity in infected children was 63%, as compared to 95.7% for HpStAR stool assay. The ClariRes correctly identified susceptible/resistant strains in all cases when compared to Etest. In a letter to the journal, Makristathis and Hirschl [50] point out that the low sensitivity could be due to the handling of the feces samples and/or to absence of duplicate testing.

Noguchi et al. amplified the 23S rRNA gene of *H. pylori* using a nested PCR on DNA extracted from stools. When sequenced, it showed that 20.4% of patients were carrying mixture of wild-type and resistant mutants for clarithromycin [51].

Antibody Detection

A novel multiplex high-throughput electrokinetically controlled immunoassay was evaluated in Canada [52]. The assay accurately distinguished between *H. pylori*-positive and -negative sera and both antibodies could be detected simultaneously. Several serologic kits were evaluated in Bangladesh [53]: 1, AccuBind™, 2, Assure© *H. pylori* Rapid Test, with a current infection marker (CIM), and 3, Helicoblot 2.1. The Immunoblot with CIM was found to be the most accurate assay, having a sensitivity of 98.3% and a specificity of 90.4%, respectively. A study from China [54] evaluated the Assure© *H. pylori* Rapid Test with CIM and found a sensitivity and specificity of 93.2% and 90.5%, respectively. The performance of the assay was poor 3 months post-treatment but increased to a sensitivity and specificity of 85.7% and 96.9%, respectively, 6 months post-treatment. An office-based serologic assay (Signify *H. pylori* test) showed a sensitivity of 52.7% and a specificity of 87.9%, respectively, in a study from Hong Kong [55].

A validated in-house ELISA based on local strains was compared to RUT, histology, culture and an in-house PCR in 40 children with recurrent abdominal pain from Mexico [56]. The authors found a lower seroprevalence in children than in adults and serology had the best diagnostic utility. A Trinity Biotec ELISA was used for a seroprevalence study in Iran in 386 children and 200 adults and found the seroprevalence of *H. pylori* infection significantly higher in adults (67.5%) than in children (46.6%) and in males as compared to females [57]. Prevalence of anti-CagA antibodies was the same in children and adults but higher in males than in females in both age groups. A study from South Korea [58] used two validated ELISA kits to study the seroprevalence of *H. pylori* infection in 8020 healthy subjects and found it to be 59.6%, i.e. significantly lower

than the 66.9% found in a similar survey in 1998. Prevalence was significantly higher in 50- to 59-year olds, of low income, males, and from the provinces.

The seroprevalence of *H. pylori* infection was 75.4% in adults from Mexico [59]. In an adjusted model, consumption of yoghurt once or more than once a week was associated with a significant decrease of infection (OR = 0.57 and 0.45, respectively) with a $p = .01$ for trend. A Dutch study in adults [60] compared serology and histology in diagnosis of advance gastric body atrophy. Concordance between histology and serology varied between 95% and 100%. A Scandinavian study [61] with an in-house Western blot assay compared the banding pattern of 115 gastric cancer patients and 110 without cancer and found that cancer patients had significantly more often antibodies to the 94 kDa protein and to the 30 kDa (UreA) as well as a combination of antibodies to the 85 kDa (VacA) and the 120 kDa (CagA).

The question "Which test is best for *Helicobacter pylori*?" was addressed in a cost-effectiveness model [62]. The outcome of the mathematical modeling was that of 1000 virtual patients, serology would have 903, urea breath test 961, and the antigen stool test 968 true positives. A study from the UK addressed patients' attitudes to *H. pylori* UBT, stool antigen test, and serology [63]. Of adults who had had a previous serologic test, 77% would prefer UBT and stool test if told that they were much more accurate than serology. Between these two tests, 58% preferred the UBT while 34% favored the stool test.

Conflicts of interest

The authors have declared no conflicts of interest.

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1. INTRODUZIONE

1.1. Obiettivo del lavoro

Scopo dello stage è stato verificare se la diagnosi di gastrite effettuata tramite la procedura del Gastropanel è sovrapponibile a quella effettuata mediante biopsia gastrica in un gruppo di pazienti che avessero effettuato entrambi gli esami. Secondariamente si è verificata la capacità del Gastropanel di identificare la presenza dell'*Helicobacter Pylori* utilizzando come esami di confronto la biopsia gastrica, l'esame ^{13}C -Urea Breath Test ed Helf (ricerca del batterio nelle feci).

I dati utilizzati provengono dai risultati del Gastropanel effettuati presso Data Medica Padova S.p.A. da marzo 2005 a dicembre 2007 e dai risultati degli esami sopra citati, eventualmente effettuati dagli stessi nello stesso periodo.

Si è inoltre analizzata la casistica dei risultati del Gastropanel.

1.2. Malattie dell'apparato Gastroenterico

La gastrite è un termine che un tempo era utilizzato impropriamente dalla gente comune per indicare "bruciore di stomaco", e, perciò, una sindrome dispeptica (dal greco, cattiva digestione); oggi assume un nuovo significato sulla base della recente acquisizione che la gastrite antrale è attribuibile all'infezione da *Helicobacter Pylori* ed è associata all'**ulcera duodenale**.

La **gastrite acuta** è un processo infiammatorio di breve durata, che spesso si risolve senza lasciare conseguenze. La causa può essere l'ingestione di alcune sostanze irritanti per la mucosa dello stomaco, per esempio alcuni farmaci, come i FANS (Farmaci Antinfiammatori Non Steroidei), l'abuso di alcool, il fumo. Talvolta la gastrite acuta è secondaria ad infezioni intestinali. La patologia si manifesta con vomito, nausea, crampi nella parte alta dell'addome. La terapia della gastrite acuta si basa sulla somministrazione di antiacidi, spasmolitici associati al riposo e a una dieta

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leggera e adeguata. Il problema si risolve in pochi giorni, ma se i disturbi permangono è opportuno eseguire esami e controlli per valutare la situazione.

La **gastrite cronica** è caratterizzata da un processo flogistico e atrofico a carattere evolutivo e a esordio precoce. Nella maggior parte dei casi si tratta di una vera e propria malattia di origine batterica associata all'infezione di *Helicobacter Pylori*. La prevalenza della gastrite cronica aumenta con l'età e nel mondo occidentale circa il 50% della popolazione al di sopra dei 60 anni presenta tale affezione morbosa.

La gastrite cronica è nella maggior parte dei casi asintomatica, ma è associata ad un aumento rischio di carcinoma gastrico e ulcera peptica.

Helicobacter Pylori

È un batterio a forma di spirale, è dotato di un rivestimento multiplo e di un flagello unipolare e di una potente attività ureasica (su cui si basa il Breath Test); sia la forma che il flagello consentono al batterio di inserirsi nella mucosa gastrica e la sua attività ureasica gli consente di crearsi una barriera di ammonio e di ione bicarbonato, essenziale per la sua colonizzazione, permette infatti di tamponare l'acidità dello stomaco che altrimenti lo ucciderebbe.

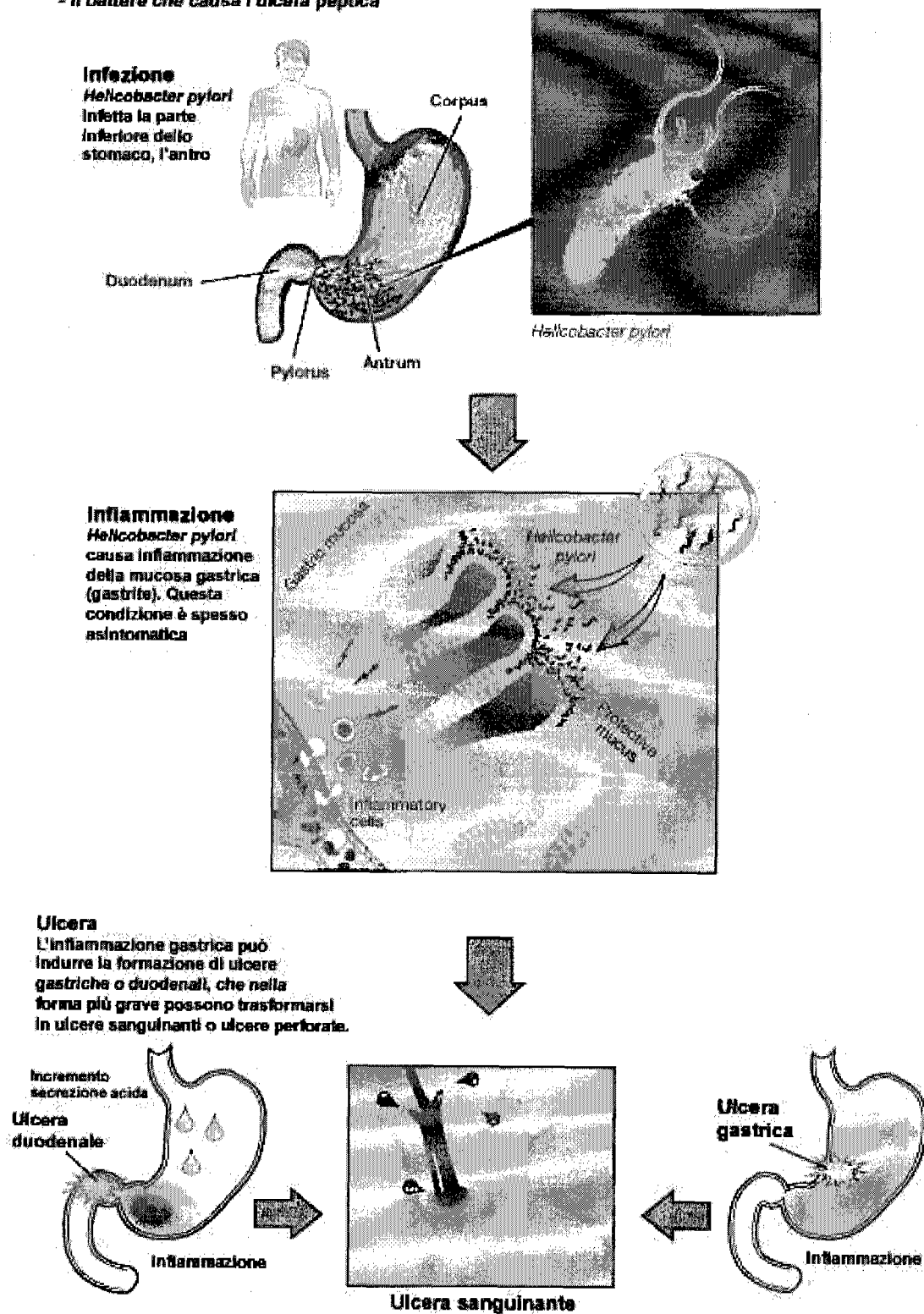
L'*Helicobacter Pylori* ha una diffusione inversamente correlata con lo sviluppo socioeconomico, infatti nei paesi sottosviluppati l'infezione è presente nel 80-90% dei soggetti.

L'*Helicobacter Pylori* si associa alla **gastrite cronica antrale** e, successivamente, i soggetti affetti da tale patologia possono avere recidive ulcerose; inoltre l'*Helicobacter Pylori* può aumentare i livelli di gastrina-17 e, quindi, la produzione di acido cloridrico, forse attraverso anche una inibizione della somatostatina. L'infezione, infine, può portare nel tempo ad un'eccessiva produzione di acido e, di conseguenza, al danneggiamento della mucosa duodenale, che in ultimo dà luogo ad una trasformazione in metaplasia gastrica intestinale (cioè isole di mucosa gastrica nel duodeno). Da qui la duodenite ed un'eventuale ulcera duodenale.

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Helicobacter pylori

- il battere che causa l'ulcera peptica



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Figura 1: Schema che illustre le localizzazione e gli effetti della presenza *Helicobacter Pylori* nella mucosa gastrica

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Epidemiologia

Il 70% della popolazione adulta soffre di bruciori di stomaco; il 50% degli uomini oltre i 60 anni soffre di gastrite cronica; il 10% soffre di ulcera peptica. La lesione peptica, singola o multipla, è più frequente nel sesso maschile che in quello femminile, in soggetti fra i 20 e i 40 anni. Trent'anni fa il rapporto tra il numero di uomini e di donne che avevano l'ulcera era di 20 a 1, oggi tale proporzione s'è ridotta a 2 a 1 a causa dell'emancipazione femminile e dell'aumentata diffusione del fumo tra le donne.

1.3. Metodi diagnostici

Le gastriti possono essere diagnosticate con metodiche invasive, che richiedono l'endoscopia e l'effettuazione di prelievi biotici (esame istologico, test rapido all'ureasi, coltura, reazione polimerasi a catena) e tecniche non invasive come l'Urea Breath Test (test del respiro), la ricerca degli antigeni del batterio su campioni fecali, o la ricerca di anticorpi specifici nel sangue e nelle urine. Tra i test non invasivi, solo il test sul respiro o la ricerca degli antigeni del batterio su campione fecale, permettono di individuare l'infezione in "atto" ed è per questo che sono chiamati attivi.

La ricerca degli anticorpi specifici rappresenta solo un indice di avvenuta esposizione all'*Helicobacter Pylori*.

1.3.1. Gastrosopia

Gastrosopia significa "guardare nello stomaco". L'esame viene eseguito con uno strumento detto gastroscopio. La Gastrosopia serve a scoprire e a volte a trattare ulcere, infiammazioni o tumori. La Gastrosopia si effettua introducendo nello stomaco, attraverso la bocca o il naso, un tubo sottile e flessibile con all'estremità una piccola telecamera.

La sonda attraversa l'esofago poi raggiunge lo stomaco. Questo esame è molto invasivo, ed i pazienti spesso rifiutano di farlo.

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Durante la Gastrosopia in genere si prelevano pezzettini di mucosa che poi possono essere analizzati mediante l'istologia (= biopsie gastriche). Poiché questi pezzi sono piccoli, può succedere che si preleva un pezzo che non contiene il batterio anche se il soggetto è stato infettato.

1.3.2. Gastropanel

Il Gastropanel è nato per rispondere all'esigenza di disporre di un esame più semplice e non invasivo come la gastroscopia. Il Gastropanel si effettua con un semplice prelievo di sangue e, oltre alla presenza di *Helicobacter Pylori*, permette di valutare una serie di marcatori (pepsinogeno I, pepsinogeno II e gastrina-17) che identificano il danno presente a livello della mucosa gastrica.

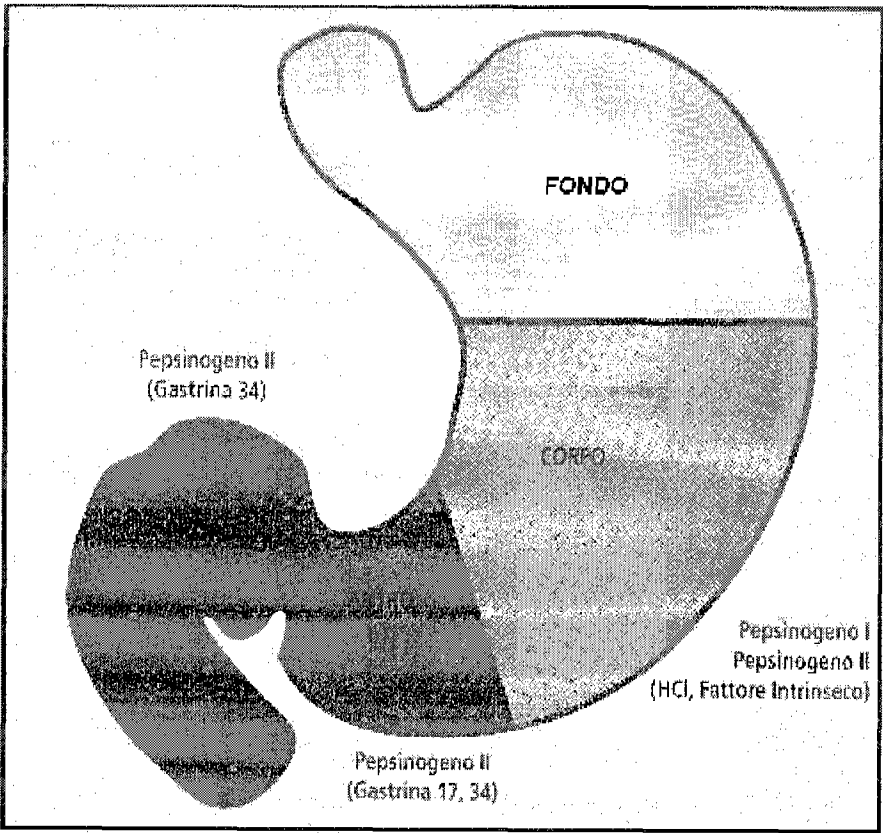


Figura 2: Localizzazione dei Marker esaminati nel Gastropanel nelle varie parti dello stomaco

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I quattro parametri valutati da Gastropanel sono:

- **PEPSINOGENO I:** è il precursore dell'enzima pepsina, viene prodotto dalle cellule della zona "alta" dello stomaco (tecnicamente corpo-fondo). Esiste una correlazione tra la diminuzione di queste cellule ed il livello del precursore nel sangue.
- **PEPSINOGENO II:** è ubiquitario, cioè prodotto da tutto lo stomaco e dal duodeno. Quando la gastrite atrofica del corpo-fondo peggiora, i livelli di pepsinogeno I e il rapporto pepsinogeno I e II decrescono.
- **GASTRINA-17:** è un ormone prodotto dalle cellule della parte "bassa" dello stomaco (antro), è un indice di danni della mucosa di questa zona dell'organo. La sua liberazione è regolata da un meccanismo a feed-back negativo ai livelli di pH gastrico e di pepsinogeni. Una concentrazione ridotta di gastrina-17, ad esempio, indica un danno gastrico antrale, mentre un suo incremento testimonia il tentativo di stimolare la produzione di HCl in caso di gastrite atrofica.
- **IgG ANTI-*HELICOBACTER PYLORI*:** le gastriti sono quasi sempre causate da infezioni da *Helicobacter Pylori*. La presenza del batterio induce la formazione di anticorpi (IgG anti-*Helicobacter Pylori*) che si ritrovano nel sangue per lunghissimi periodi, anche dopo essere stati sottoposti a trattamento per eradicare il virus.

La risposta diagnostica del Gastropanel è codificata come segue:

- Gastrite Atrofica dell'antro (A)
- Gastrite Atrofica dell'antro e del corpo (AC)
- Gastrite Atrofica del corpo (C)
- Gastrite non atrofica (S)
- Normale (N)

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La figura 3 riassume l'algoritmo utilizzato per la determinazione della codifica.

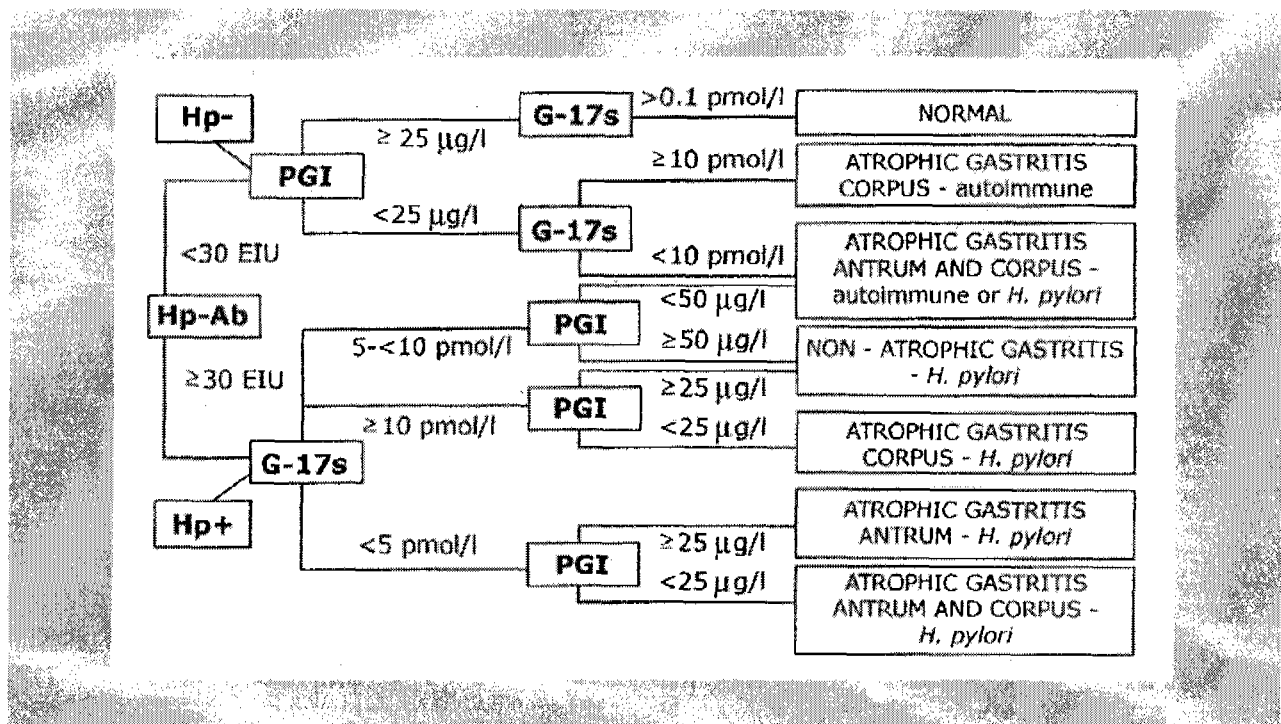


Figura 3: Parametri misurati nel Gastronpanel: interpretazione dei risultati e valori di riferimento

1.3.3. ¹³C-Urea Breath Test

Il **Breath test** , o "test del respiro", è un esame non invasivo grazie al quale si può diagnosticare la presenza del batterio *Helicobacter Pylori* nella mucosa gastrica. E' una metodica rapida, riproducibile ed economica che si basa sulla capacità del microbo di scindere rapidamente l'urea (ureasi) somministrata per bocca, fino a ottenere acqua e anidride carbonica. Il test è in grado di valutare la carica batterica presente nello stomaco intero, ovviando alla distribuzione irregolare del batterio sulla mucosa gastrica, ed ha una sensibilità ed una specificità molto elevate, di circa il 98%.

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2. METODI

2.1. La “bontà” di un test diagnostico

La sovrapposibilità delle metodiche è stata valutata mediante tre indici: l’accuratezza, la specificità e la sensibilità.

Il risultato di un test diagnostico può facilmente essere riportato in forma dicotomica (Positivo – Negativo), volendo confrontare due metodiche una affermata definita come gold standard con una in fase sperimentale è possibile costruire una tabella con l’incrocio dei possibili risultati.

Esame	Golden Standard (presenza della malattia)		
	VERO	FALSO	TOTALE
POSITIVO	a	b	a+b
NEGATIVO	c	d	c+d
TOTALE	a+c	b+d	n

In questa tabella:

- a indica il numero di soggetti positivi effettivamente ammalati (Veri Positivi)
- c indica il numero di soggetti negativi al test ma effettivamente ammalati (Falsi Negativi)
- b indica il numero di soggetti positivi al test ma in realtà sani (Falsi Positivi)
- d infine indica il numero di soggetti negativi al test ed effettivamente sani (Veri Negativi)

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L'accuratezza misura la capacità del test di effettuare una diagnosi correttamente: diagnosi corrette sono (a) il numero di Veri Positivi e (d) il numero Veri Negativi, l'accuratezza è pertanto stimata dal rapporto $(a+d)/n$. Il test ideale ha un'accuratezza del 100% cioè b e c sono nulli: tutte le diagnosi sono corrette

La sensibilità è la capacità di identificare correttamente i soggetti ammalati. Misura quindi la capacità del test di individuare come positivo un soggetto effettivamente ammalato. Nella tabella i soggetti ammalati sono rappresentati da $(a+c)$ e, fra questi, i positivi al test sono rappresentati da (a); quindi, la sensibilità si calcola con la proporzione $a/(a+c)$. Il test che non ha Falsi Negativi ha una sensibilità del 100%.

La specificità è la capacità di identificare correttamente soggetti sani. Misura quindi la capacità del test di individuare come negativo un soggetto sano. Nella tabella i soggetti sani sono rappresentati da $(b+d)$ e, fra questi, i negativi al test sono i soli (d); quindi, la specificità si calcola con la proporzione $d/(b+d)$. Il test che non presenta Falsi Positivi ha una specificità del 100%.

Un test di screening dovrebbe garantire di identificare tutti i soggetti malati quindi di non presentare Falsi Negativi perciò deve avere una sensibilità molto elevata.

2.2. La preparazione dei dati

I dati si presentavano in forma eterogenea: i risultati del Gastropanel erano stati raccolti in un foglio Excel con l'identificativo del paziente, la data di esecuzione dell'esame, i valori dei parametri rilevati nel sangue, la presenza l'*Helicobacter Pylori* ed infine la classe di diagnosi a cui il paziente era stato attribuito.

Gli altri esami erano archiviati nel sistema informativo aziendale in forme molto diverse. Le biopsie riportavano per intero la diagnosi, l'Helf l'indicazione POSITIVO/NEGATIVO mentre il Breath Test, eseguito presso altri laboratori, disponeva della risposta solo su supporto cartaceo.

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La prima operazione ha riguardato la casistica del Gastropanel: sono stati identificati i singoli pazienti attribuendo a ciascuno uno o più risultati a seconda del numero di volte che avevano eseguito l'esame.

Sulla base dei pazienti del Gastropanel sono stati estratti i risultati degli esami (Helf, Biopsie e Breath Test) che gli stessi soggetti avevano eseguito nello stesso periodo. Per il Breath Test si è recuperata la risposta cartacea ed è stata inserita informatizzata. Le Biopsie sono state classificate, con l'aiuto di un medico, mediante la ricerca di parole chiave nel testo della risposta e attribuendo ad esse la codifica del Gastropanel. Questa operazione ha sicuramente comportato un grosso errore di classificazione in quanto la risposta della biopsia non è pensata per fornire da sola la diagnosi ma va ad integrare la gastroscopia il cui risultato non è disponibile.

È stato infine generato un foglio elettronico che in ogni riga riportava i risultati del Gastropanel e dell'altro esame eseguito. Nel caso di pazienti con più di un Gastropanel o con più esami di confronto, l'associazione è stata eseguita sulla base delle date di esecuzione associando al Gastropanel ogni altro esame eseguito e nel caso di esami multipli quelli con la data più vicina.

Infine, per garantire che le condizioni del paziente fossero le stesse tra esecuzione del Gastropanel e di un altro esame, sono state selezionate solo le coppie in cui la data di esecuzione differivano per non più di 45 giorni. Anche questa scelta, necessaria per poter confrontare esami, ha introdotto una fonte di errore nella valutazione della presenza di *Helicobacter Pylori*: il trattamento dura meno di 45 giorni ed è pertanto possibile che pazienti positivi al Gastropanel avessero eradicato il batterio al momento dell'esame di controllo.

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La risposta diagnostica del Gastropanel è codificata come segue:

- Gastrite Atrofica dell'antro (A)
- Gastrite Atrofica dell'antro e del corpo (AC)
- Gastrite Atrofica del corpo (C)
- Gastrite non atrofica (S)
- Normale (N)

Di seguito è riportata la distribuzione delle diagnosi di tutte le visite, il 65,9% delle valutazioni risulta normale.

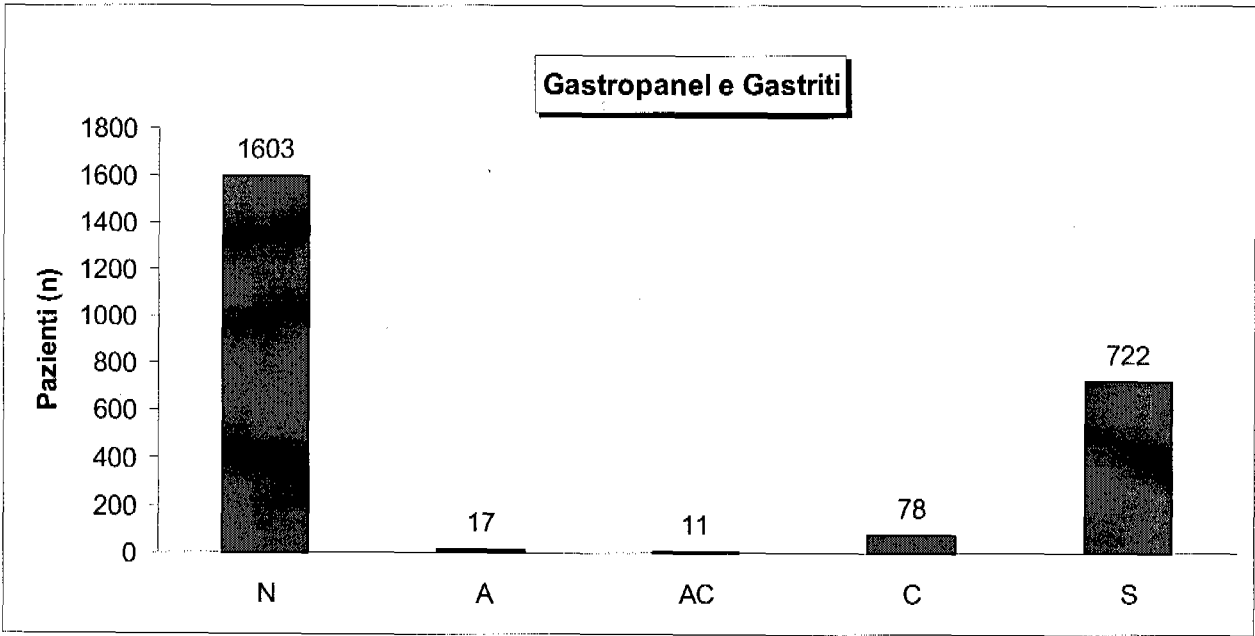


Figura 2: Presenza di gastrite nei pazienti analizzati. N = soggetti sani, A = soggetti con gastriti dell'antro, AC = soggetti con gastriti dell'antro e del corpo, C = soggetti con gastriti del corpo, S = soggetti con assenza gastrite

27
27
225

Si è poi valutata l'interazione tra Helicobacter Pylori e tipo di gastrite.

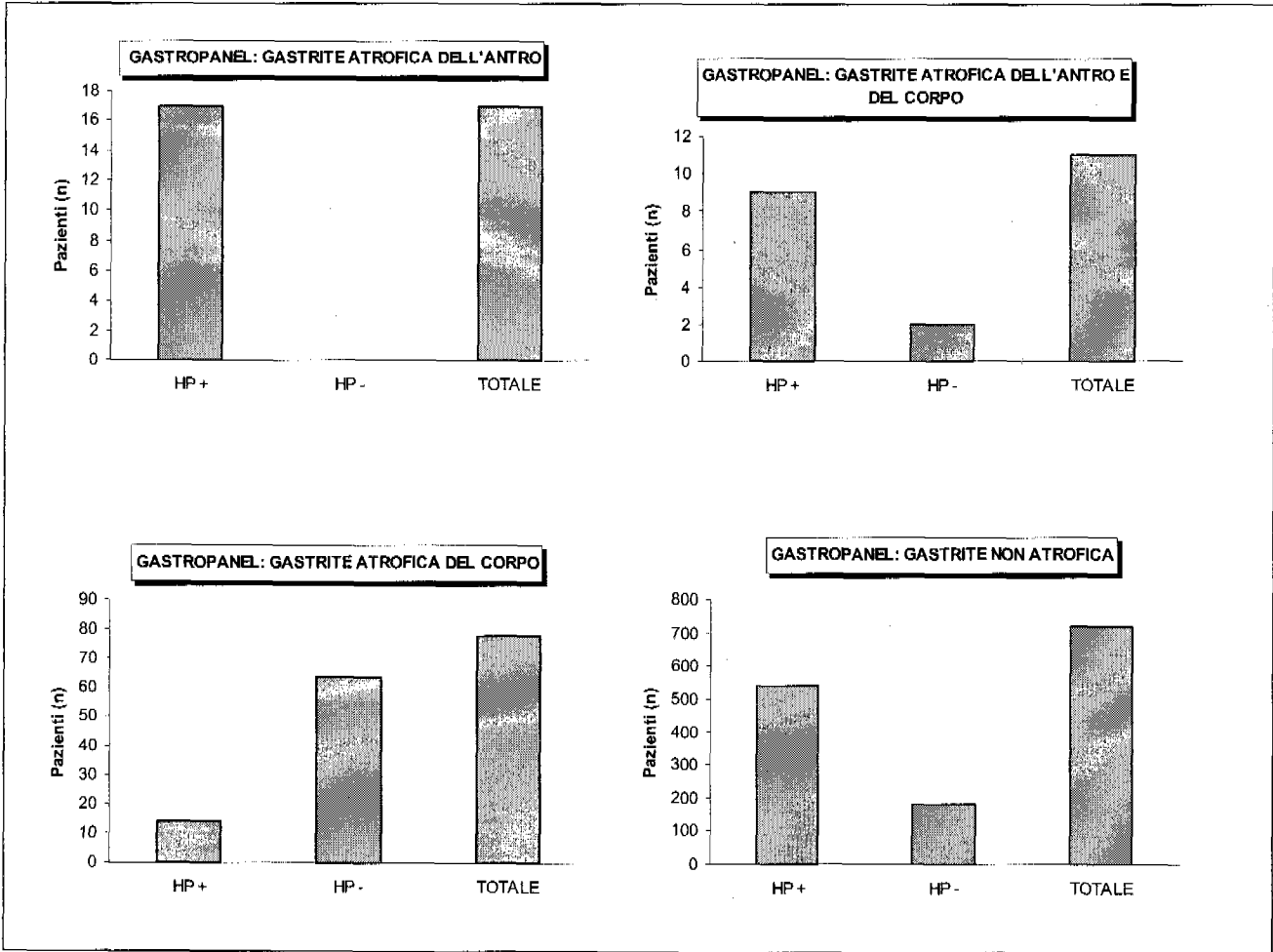


Figura 3: Presenza dell'Helicobacter Pylori nelle diverse forme di gastrite

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3.2.3. Confronto tra Gastropanel e Biopsie Gastriche

	Presenza Helicobacter Pylori (Biopsie Gastriche)		
Gastropanel	VERO	FALSO	TOTALE
POSITIVO	52	11	63
NEGATIVO	32	129	161
TOTALE	84	140	224

Accuratezza	0,808	0,756 - 0,860
Sensibilità	0,619	0,515 - 0,723
Specificità	0,921	0,877 - 0,966

Tra gli esami presi in considerazione la biopsia gastrica è il meno specifico infatti, poiché l'esame cerca fisicamente la presenza del batterio nel campione, la determinazione della presenza dipende dalla probabilità di aver prelevato un campione di mucosa gastrica effettivamente contaminato.

La numerosità è maggiore l'accuratezza risulta buona sempre molto scarsa la sensibilità. È da ricordare che il Gastropanel misura i livelli di immunoglobuline nel sangue che dopo eradicazione del batterio rimangono molto alte per lungo tempo determinando la possibile causa dell'alto numero di falsi negativi.

La probabilità di presenza della patologia se l'esame è positivo è 0,825 (82,5%).

La probabilità di assenza della patologia se l'esame è negativo è 0,801 (80,1%).

AA
231

3.3. Confronto tra la capacità diagnostica del Gastropanel e biopsia gastrica

Per confrontare il risultati diagnostici del Gastropanel con le biopsie gastriche si è proceduto a codificare tutte le diagnosi delle biopsie mediante la ricerca di parole chiave nel testo. Purtroppo non è stato possibile, nei referti, distinguere la sede delle lesioni. Pertanto tutte le diagnosi di gastrite atrofica sono state accorpate in una sola modalità.

Gastropanel	Biopsie Gastriche			
	Atrofico	Non Atrofico	Normale	TOTALE
Atrofico	5	1	5	11
Non Atrofico	54	0	19	73
Normale	21	2	117	140
TOTALE	80	3	141	224

L'accuratezza è stata valutata accorpendo la modalità "non atrofico" (che rappresenta una lieve lesione) prima con i normali e quindi con le gastriti.

- ATROFICO vs (NON ATROFICO + NORMALE)

Accuratezza	0,638	0,575-0,701
Sensibilità	0,063	0,009-0,116
Specificità	0,958	0,926-0,991

Accorpendo la gastrite non atrofica ai normali il risultato è pessimo: l'accuratezza è molto bassa e la specificità pessima.

L'indicazione è comunque che il Gastropanel sia specifico per l'individuazione della gastrite. Raramente un risultato normale si dimostra errato.

4/24/23

4. CONCLUSIONI

Il campione considerato, persone che si sono rivolte ad un laboratorio analisi possibilmente per sintomi legati alla gastrite per effettuare il Gastropanel, non presenta caratteristiche tali da rappresentare la popolazione. In più il fatto che abbiano ripetuto un altro esame sempre legato alla diagnostica di problemi relativi all'apparato gastroenterico aggiunge un ulteriore criterio di diversificazione rispetto alla popolazione.

Per questa ragione il risultati sono da interpretare come un'indicazione o una conferma rispetto a quanto riportato in letteratura.

Come del resto atteso, il Gastropanel non è un esame specifico per la ricerca dell'*Helicobacter Pylori* e mostra anche dei limiti (la bassa sensibilità) come esame di screening. Questo risultato trova anche una conferma nel fatto che, le ultime versioni del software Gastrosoft distribuito dalla BioHit proprietaria del brevetto, utilizza informazioni esterne al prelievo di sangue per valutare la presenza di *Helicobacter Pylori*. La stessa versione (disponibile nel sito <http://www.gastropanel.net>) distingue le due situazioni di valutazione prima o dopo l'eradicazione del batterio.

Dal punto di vista della concordanza nella diagnosi si riportano di seguito i risultati ottenuti in uno studio finlandese su 404 pazienti affetti da dispepsia sottoposti ad entrambe le procedure per la diagnosi di gastrite.

Diagnosis of atrophic gastritis vs histology, N=404	Gastropanel
Overall accuracy	81% (77-85%)
Sensitività	79% (69-89%)
Specificità	91% (88-94%)

SD
24/11
235

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Stg
230
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(54) Título: **Método y kit para la detección de *Helicobacter pylori*.**

(57) Resumen:

El método se basa en el ensayo del aliento con urea marcada con carbono-13 y que comprende (a) administrar al paciente una solución acuosa de ácido cítrico con un pH comprendido entre 2 y 2,5; (b) recoger una muestra del aliento del paciente para determinar el contenido basal en carbono-13; (c) administrar al paciente una cantidad apropiada de urea marcada con carbono-13; (d) recoger una muestra del aliento del paciente para determinar el contenido en carbono-13 postadministración de la urea marcada; y (e) analizar las muestras de aliento recogidas para determinar el contenido en carbono-13 antes y después de la administración de urea marcada con carbono-13. El kit contiene un recipiente para el ácido cítrico, otro para la urea marcada con carbono-13, unos exetainers destinados a contener el aliento del paciente, y unos medios para soplar el aliento dentro de los exetainers.

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La Figura 2 muestra el dibujo de un exeteiner del tipo mostrado en la Figura 1, con todos sus componentes acoplados.

Descripción detallada de la invención

1. Método para la detección de *Helicobacter pylori*

En un primer objeto de esta invención, se proporciona un método para la detección de *Helicobacter pylori*, basado en el ensayo del aliento con urea marcada con carbono-13, que comprende las etapas generales de:

- administrar al paciente una cantidad apropiada de urea marcada con carbono-13; y

- determinar el contenido en carbono-13 a partir de: muestras de aliento del paciente antes y después de la administración de urea marcada con carbono-13;

que se caracteriza porque:

- se administra al paciente una solución acuosa de ácido cítrico que comprende ácido cítrico en una cantidad suficiente para dar un pH comprendido entre 2 y 2,5;

- el contenido en carbono-13 en la muestra de aliento del paciente antes de la administración de urea marcada con carbono-13 se puede realizar antes de, o alternativamente después de, administrar al paciente dicha solución acuosa de ácido cítrico; y

- la urea marcada con carbono-13 puede ser administrada al paciente después de, o simultáneamente junto con, la administración de dicha solución acuosa de ácido cítrico.

Una primera alternativa de realización del método para la detección de *Helicobacter pylori*, basado en el ensayo del aliento con urea marcada con carbono-13, proporcionado por esta invención implica la administración al paciente de la solución acuosa de ácido cítrico antes de determinar el contenido basal en carbono-13 en la muestra de aliento y antes de administrar la urea marcada con carbono-13, y comprende las etapas de:

- a) administrar al paciente una solución acuosa de ácido cítrico que comprende ácido cítrico en una cantidad suficiente para dar un pH comprendido entre 2 y 2,5;

- b) recoger una muestra del aliento del paciente con el fin de determinar el contenido basal en carbono-13;

- c) administrar al paciente una cantidad apropiada de urea marcada con carbono-13;

- d) recoger una muestra del aliento del paciente con el fin de determinar el contenido en carbono-13 tras la administración de la urea marcada con carbono-13; y

- e) analizar las muestras de aliento recogidas para determinar el contenido en carbono-13 antes y después de la administración de urea marcada con carbono-13.

La solución acuosa de ácido cítrico comprende una disolución en agua de ácido cítrico en cantidad suficiente para dar un pH comprendido entre 2 y 2,5. Normalmente, ese pH se alcanza con una disolución acuosa de ácido cítrico a una concentración de, aproximadamente, 0,021 g/ml. El empleo de esta solución cumple dos objetivos, por una parte, contribuye al retardamiento del vaciamiento gástrico, con lo que se consigue que la urea marcada con carbono-13 permanezca más tiempo

en el antro estomacal, y, por otra parte, al rendir un pH como el del antro estomacal, permite que la colonia de *H. pylori* pueda ejercer con mayor seguridad su actividad ureasa hidrolizando la urea en dióxido de carbono y urea. El empleo de esta solución es fundamental y crítico para que el ensayo sea significativamente eficaz, sensible, fiable y reproducible, y constituye uno de los aspectos esenciales de esta invención.

La solución acuosa de ácido cítrico puede contener, además, unos aditivos alimentarios o farmacéuticos aceptables, que mejoren sus propiedades y características organolépticas tales como, edulcorantes, aromatizantes, colorantes y conservantes.

Dicha solución acuosa de ácido cítrico puede obtenerse fácilmente por disolución de los componentes en la cantidad apropiada de agua. En una realización particular y preferida del método objeto de esta invención, la solución acuosa de ácido cítrico se obtiene disolviendo 4,2 g de ácido cítrico en 200 ml de agua.

El empleo de una solución acuosa de ácido cítrico que comprende ácido cítrico en cantidad suficiente para dar un pH comprendido entre 2 y 2,5, opcionalmente junto con otros aditivos alimentarios o farmacéuticos aceptables, en la realización de un método de detección de *H. pylori*, basado en el ensayo del aliento con urea marcada con carbono-13, constituye un objeto adicional de esta invención.

Después de la administración de la solución acuosa de ácido cítrico se recogen muestras del aliento del paciente en unos recipientes adecuados, tal como unos exeteiners, al objeto de determinar el contenido basal en carbono-13. Como es conocido, el carbono-13 es un isótopo que normalmente está, presente en cantidades muy pequeñas en productos de origen natural, tal como la urea procedente del metabolismo proteico. En general, los átomos de carbono presentes en una muestra de urea de origen natural comprenden mayoritariamente el isótopo carbono-12 y una muy pequeña cantidad del isótopo carbono-13 (aproximadamente el 1,1%). Normalmente las muestras se recogen por duplicado al cabo de unos 10 minutos tras la administración de la solución acuosa de ácido cítrico. Los recipientes adecuados son, preferiblemente, unos exeteiners del tipo mostrado en las Figura 1 y 2, cuyas características se mencionan con más detalle al tratar el kit proporcionado por esta invención. En una realización particular y preferida del método objeto de esta invención, el paciente introduce su aliento en los exeteiners por soplado con ayuda de una pajita previamente introducida en ellos y posteriormente se cierra el exeteiner por medio de su tapón.

A continuación, se administra al paciente una cantidad apropiada de urea marcada con carbono-13. El término "cantidad apropiada" se refiere a una cantidad segura y efectiva de urea marcada con carbono-13 que es suficiente para producir un nivel detectable de dióxido de carbono marcado con carbono-13 sin provocar efectos adversos, tales como toxicidad, o reacciones alérgicas, en el paciente.

La urea marcada con carbono-13 se adminis-

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carbono-13 se recogen muestras del aliento del paciente en unos exeteiners con el fin de determinar el contenido en carbono-13 post-administración, de la urea marcada con dicho isótopo. En general, las muestras se recogen por duplicado después de dejar actuar la actividad ureasa durante un periodo de tiempo adecuado, normalmente unos, 30 minutos. El paciente introduce su aliento en los exeteiners mediante soplado con ayuda de una pajita tal como se ha mencionado previamente.

Los exeteiners con las muestras de aliento recogidas, pueden mantenerse a temperatura ambiente durante varios meses, sin que el contenido del aliento del paciente se vea afectado adversamente.

Las muestras de aliento recogidas en los exeteiners se envían al laboratorio para efectuar el análisis del aliento y determinar el contenido en carbono-13 antes y después de la administración de urea marcada con carbono-13, mediante el empleo de técnicas apropiadas, tales como cromatografía de gases y espectroscopia de masas.

En general, para la ejecución del método para la detección de *H. pylori* objeto de esta invención, en cualquiera de sus alternativas de realización, es conveniente que el paciente se encuentre en ayunas, siendo recomendable que el paciente no haya ingerido ningún tipo de alimento en las 6-8 horas previas a la realización del ensayo.

La comparación entre el contenido basal en carbono-13 y el contenido en carbono-13 tras la administración de urea marcada con carbono-13 permite evaluar la actividad ureasa. Una actividad ureasa alta, baja o nula (punto de corte o de referencia [cut-off]: 3-5) puede ser indicativa de la presencia de un microorganismo con actividad ureasa, tal como el *H. pylori* y, por tanto, puede ser indicativo de la existencia de una enfermedad gastroduodenal.

Por consiguiente, una aplicación particular del método de detección de *H. pylori* proporcionado por esta invención consiste en el diagnóstico de enfermedades gastroduodenales mediante un método no invasivo basado en el test del aliento con urea marcada con carbono-13. Por tanto, el método de detección de *H. pylori* proporcionado por esta invención puede ser utilizado en el diagnóstico de enfermedades gastroduodenales, tales como úlcera péptica duodenal, úlcera duodenal, úlcera gástrica, dispepsia, linfomas "no Hodgkins" gástricos e incluso en el cáncer de estómago.

Otra aplicación del método de detección de *H. pylori* objeto de esta invención es la de comprobar la evolución del tratamiento de la enfermedad gastroduodenal y, en particular, la erradicación del *H. pylori*, puesto que permite determinar la erradicación del *H. pylori* hasta en un plazo máximo de un mes después del tratamiento sin dar lugar a falsos negativos como ocurre con los métodos serológicos.

El método de detección de *H. pylori* proporcionado por esta invención, en cualquiera de sus alternativas de realización, presenta numerosas ventajas sobre otros métodos similares conocidos ya que:

- es un método no invasivo, por lo que no requiere la realización de una gastroscopia en el pa-

ciente;

- es seguro, puesto que utiliza un isótopo estable y no radiactivo (carbono-13) en cantidades moderadas;

- es fiable y reproducible, puesto que reproduce las condiciones estándar del entorno del antro estomacal y asegura la pervivencia de la posible colonia *H. pylori*;

- es eficaz, puesto que refleja la infección de toda la superficie estomacal;

- es rápido, ya que proporciona resultados en 48 horas;

- es fácil, sencillo, no requiere personal cualificado y, de hecho, puede realizarse tanto en la propia consulta o clínica del médico como en el propio domicilio del paciente y enviar los recipientes cerrados conteniendo las distintas muestras de aliento al laboratorio de análisis;

- es cómodo, puesto que el paciente no está obligado a mantener una determinada posición durante el desarrollo del ensayo, de hecho, durante la realización del ensayo el paciente no necesita mantenerse en posición horizontal, como en otros ensayos del estado de la técnica, sino que puede cambiar de postura y permanecer sentado o de pie, siendo la única limitación la de que durante la realización del ensayo el paciente no puede fumar ni ingerir ninguna comida ni bebida;

- la recogida del aliento es muy sencilla y económica; y

- tiene una amplia aplicación, puesto que puede ser aplicado en mujeres embarazadas, niños, adultos y ancianos.

2. Kit para la detección de *H. pylori*

En un segundo objeto, se proporciona un kit para la detección de *Helicobacter pylori*, en particular, un kit para la realización del método basado en el test del aliento con urea marcada con carbono-13 previamente descrito. En su forma más simple, el kit comprende un estuche que contiene:

- un recipiente que contiene urea marcada con carbono-13;

- al menos, dos recipientes destinados a contener el aliento del paciente, uno de los cuales está destinado a contener el aliento del paciente tras la administración del ácido cítrico pero antes de la administración de la urea marcada con carbono-13, mientras que el otro recipiente está destinado a contener el aliento del paciente tras la administración de la urea marcada con carbono-13, estando dichos recipientes constituidos por unos tubos de cristal provistos de unos tapones que van a rosca y tienen un septum; y

- unos medios para soplar el aliento dentro de los recipientes antes citados.

En una realización particular de esta invención, el kit incluye, además, un recipiente que contiene ácido cítrico, el cual puede encontrarse en estado sólido, en una cantidad tal que tras disolución en la cantidad apropiada de agua rinde un pH comprendido entre 2 y 2,5. En este caso particular, el recipiente que contiene el ácido cítrico es un sobre o similar. Alternativa y opcionalmente, el recipiente puede contener además del ácido cítrico unos aditivos alimentarios o farmacéuticos aceptables (edulcorantes, aromatizantes, conservantes y colorantes). Por tanto, en una

realización particular y preferida del kit objeto de esta invención se proporciona un sobre que contiene 4,2 g de ácido cítrico, opcionalmente junto con otros aditivos, para su disolución en 200 ml de agua.

El recipiente que contiene la urea marcada con carbono-13, puede ser un sobre conteniendo la cantidad apropiada de urea marcada con carbono-13 en forma de polvo, o un blister conteniendo un comprimido de urea marcada con carbono-13 con la dosis apropiada, o, alternativamente, un frasco cerrado que contiene la cantidad apropiada de urea marcada con carbono-13 y el frasco tiene el volumen adecuado para obtener la disolución acuosa de urea marcada con carbono-13 a la concentración deseada. En una realización particular, el recipiente que contiene la urea marcada con carbono-13 es un frasco de, al menos, 20 a 50 ml, que contiene entre 75 y 100 mg de urea marcada con carbono-13 en polvo. En otra realización particular, dicho recipiente es un blister que contiene un comprimido, opcionalmente dispersable, que contiene entre 75 y 100 mg de urea marcada con carbono-13.

Los recipientes destinados a contener el aliento del paciente son unos exeteiners como los que se muestran en las Figuras 1 y 2, donde puede apreciarse que dichos exeteiners están constituidos por un tubo (1), preferentemente de cristal, cuyos tapones (2) van a rosca y tienen un septum (3) en el centro, hecho de un material elastómero apto para farmacia, que permite su penetración por medio de una aguja que recoge el aire contenido en el exeteiner para su análisis posterior. El septum (3) está especialmente diseñado para que la aguja de la máquina de recogida de aire pueda pinchar y realizar su función sucesivas veces sin romperse.

Para evaluar la fiabilidad y reproducibilidad del ensayo, objeto de esta invención es conveniente recoger las muestras de aliento, tanto antes como después de la administración de urea marcada con carbono-13, por duplicado, por lo que, el kit de la invención contendrá, preferentemente, cuatro exeteiners, de los cuales dos se utilizarán para recoger el aliento del paciente tras la administración del ácido cítrico pero antes de la administración de la urea marcada con carbono-13, mientras que los otros dos recogerán el aliento del paciente tras la administración de la urea marcada con carbono-13. Para facilitar la interpretación de los resultados obtenidos es conveniente que los exeteiners lleven alguna marca que permita distinguir entre los que recogen el aliento antes de la administración de urea marcada con carbono-13 y los que recogen el aliento tras la administración de la urea marcada con carbono-13.

En general, los medios para soplar el aliento dentro de los recipientes son unas pajitas o similares.

Por tanto, en una realización particular y preferida de esta invención se proporciona un kit para la detección de *H. pylori* en particular, un kit para la realización del test del aliento con urea marcada con carbono-13 previamente descrito, que comprende un estuche que contiene:

- un sobre que contiene ácido cítrico, en estado sólido, en cantidad suficiente para que, tras diso-

lución en la cantidad adecuada de agua, rinda un pH comprendido entre 2 y 2,5, junto con opcionalmente, unos aditivos, tales como edulcorantes, aromatizantes, conservantes y colorantes;

- un frasco que contiene la cantidad apropiada de urea marcada con carbono-13 en forma de polvo, conteniendo dicho frasco el volumen necesario para disolver la urea y alcanzar la concentración deseada, o alternativamente, un blister conteniendo un comprimido con la cantidad apropiada de urea marcada con carbono-13;

- cuatro exeteiners destinados a contener el aliento del paciente, dos de los cuales se utilizan para recoger el aliento del paciente tras la administración de la solución acuosa de ácido cítrico pero antes de la administración de la solución acuosa de urea marcada con carbono-13, mientras que los otros, dos están destinados a contener el aliento del paciente tras la administración de la solución acuosa de urea marcada con carbono-13;

y - unas pajitas para soplar el aliento dentro de los exeteiners.

Ventajosamente, los exeteiners están marcados de forma que se puedan distinguir de manera sencilla los exeteiners destinados a ser utilizados para recoger el aliento antes de la administración de la urea marcada con carbono-13 de los que se utilizan para recoger el aliento después de la administración de la urea marcada con carbono-13.

Los siguientes ejemplos ilustran la invención.

Ejemplo 1

Preparación de una solución acuosa de ácido cítrico

Se prepara una solución acuosa de ácido cítrico con un pH comprendido entre 2 y 2,5, disolviendo en 200 ml de agua una mezcla formada por:

	Gramos	Porcentaje
Acido cítrico	4,2	96,1
Carbonato sódico	0,1	2,24
Aroma de limón (polvo)	0,04	0,9
Aspartamo	0,03	0,67
Sunset-Yellow	0,002	0,04
Total	4,472	99,95

Ejemplo 2

*Ensayo para la detección de *H. pylori**

Un paciente sospechoso de padecer una enfermedad gastroduodenal relacionada con *H. pylori*, se somete al ensayo que se describe a continuación. El ensayo se realiza sobre un paciente adulto, que haya permanecido 8 horas en ayuno.

Al paciente se le administran 200 ml de una solución acuosa de ácido cítrico como la preparada en el Ejemplo 1. Al cabo de 10 minutos se recogen unas muestras de aliento del paciente, por duplicado, en unos exeteiners identificados con el indicativo "BASAL" que se utilizan para determinar el contenido basal en carbono-13. Para la recogida del aliento, el paciente hace uso de una pajita previamente situada dentro del exeteiner y sopla lentamente el aire espirado hasta que aparece condensación del aliento en las paredes internas del exeteiner, y a continuación sigue espirando el aliento a la vez que retira la pajita y

REIVINDICACIONES

1. Un kit para la detección de *Helicobacter pylori*, mediante la realización de un método basado en el ensayo del aliento con urea marcada con carbono-13, que comprende un estuche que contiene:

- un recipiente que contiene urea marcada con carbono-13;

- al menos, dos recipientes destinados a contener el aliento del paciente, uno de los cuales está destinado a contener el aliento del paciente tras la administración del ácido cítrico pero antes de la administración de la urea marcada con carbono-13, mientras que el otro recipiente está destinado a contener el aliento del paciente tras la administración de la urea marcada con carbono-13, estando dichos recipientes constituidos por unos tubos de cristal provistos de unos tapones que van a rosca y tienen un septum; y

- unos medios para soplar el aliento dentro de los recipientes antes citados.

2. Un kit según la reivindicación 1, que comprende además un recipiente que contiene ácido cítrico.

3. Un kit según la reivindicación 2, en el que el ácido cítrico se encuentra en estado sólido, en una cantidad tal que tras disolución en la cantidad apropiada de agua rinde un pH comprendido entre 2 y 2,5.

4. Un kit según la reivindicación 3, en el que el recipiente que contiene el ácido cítrico es un sobre.

5. Un kit según la reivindicación 2, en el que el recipiente del ácido cítrico contiene, además del ácido cítrico unos aditivos alimentarios o farmacéuticos aceptables seleccionados del grupo formado por edulcorantes, aromatizantes, conservantes y colorantes.

6. Un kit según la reivindicación 1, en el que el recipiente que contiene la urea marcada con carbono-13 es un frasco que contiene la cantidad apropiada de urea marcada con carbono-13 y el frasco tiene el volumen adecuado para obtener la disolución acuosa de urea marcada con carbono-13 a la concentración deseada.

7. Un kit según la reivindicación 1, en el que el recipiente que contiene la urea marcada con carbono-13 es un blister que contiene un comprimido, opcionalmente dispersable, que contiene la cantidad apropiada de urea marcada con carbono-13.

8. Un kit según la reivindicación 1, en el que los recipientes destinados a contener el aliento del paciente son unos exeteiners y están constituidos por un tubo (1) de cristal, cuyos tapones (2) van a rosca y tienen un septum (3) en el centro, hecho de un material elastómero apto para farmacia, que permite su penetración por medio de una aguja para recoger el aire contenido en el exeteiner para su análisis posterior.

9. Un kit según la reivindicación 1, en el que dichos medios para soplar el aliento dentro de los recipientes son unas pajitas.

10. Un kit para la detección de *Helicobacter pylori*, mediante la realización de un método basado en el ensayo del aliento con urea marcada con carbono-13, que comprende un estuche que contiene:

- un sobre que contiene ácido cítrico, en estado sólido, en cantidad suficiente para que, tras disolución en la cantidad adecuada de agua, rinda un pH comprendido entre 2 y 2,5, junto con opcionalmente, unos aditivos, tales como edulcorantes, aromatizantes, conservantes y colorantes;

- un recipiente que contiene urea marcada con carbono-13 seleccionado entre:

i) un frasco que contiene la cantidad apropiada de urea marcada con carbono-13, conteniendo dicho frasco el volumen necesario para disolver la urea y alcanzar la concentración deseada, o alternativamente,

ii) un blister conteniendo un comprimido, opcionalmente dispersable, que contiene la cantidad apropiada de urea marcada con carbono-13;

- cuatro exeteiners destinados a contener el aliento del paciente, dos de los cuales se utilizan para recoger el aliento del paciente tras la administración de la solución acuosa de ácido cítrico pero antes de la administración de la solución acuosa de urea marcada con carbono-13, mientras que los otros dos están destinados a contener el aliento del paciente tras la administración de la solución acuosa de urea marcada con carbono-13; y

- unas pajitas para soplar el aliento dentro de los exeteiners.

11. Un kit según la reivindicación 10, en el que el dicho sobre contiene 4,3 g de ácido cítrico, opcionalmente junto con otros aditivos, para su disolución en 200 ml de agua.

12. Un kit según la reivindicación 10, en el que el frasco que contiene la urea marcada con carbono-13 es un frasco de, al menos, 20 a 50 ml y contiene entre 75 y 100 mg de urea marcada con carbono-13.

13. Un kit según la reivindicación 10, en el que dicho comprimido contiene entre 75 y 100 mg de urea marcada con carbono-13.

14. Un kit según la reivindicación 10, en el que dichos exeteiners están marcados de forma que se puedan distinguir de manera sencilla los exeteiners destinados a ser utilizados para recoger el aliento antes de la administración de la urea marcada con carbono-13 de los que se utilizan para recoger el aliento después de la administración de la urea marcada con carbono-13.

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(21) International Application Number: PCT/SE95/01212 (22) International Filing Date: 17 October 1995 (17.10.95) (30) Priority Data: 9403755-3 2 November 1994 (02.11.94) SE (71) Applicant (for all designated States except US): DIABACT AB [SE/SE]; Holmvägen 22, S-756 61 Uppsala (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): NYSTRÖM, Christer [SE/SE]; Holmvägen 22 B, S-756 61 Uppsala (SE). LUNDQVIST, Thomas [SE/SE]; Flogstavägen 146, S-752 63 Uppsala (SE). PETTERSSON, Anders [SE/SE]; Knaver- stad 13060, S-442 97 Kode (SE). (74) Agents: ONN, Thorsten et al.; AB Stockholms Patentbyrå, Zacco & Bruhn, P.O. Box 23101, S-104 35 Stockholm (SE).		(81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). ARIPO patent (KE, MW, SD, SZ, UG). Published <i>With international search report.</i> <i>With amended claims.</i> <i>In English translation (filed in Swedish).</i>
(54) Title: DIAGNOSTIC PREPARATION FOR DETECTION OF HELICOBACTER PYLORI (57) Abstract The present invention relates to a preparation for administering isotope-labelled urea together with acid in tablet form, in the form of a capsule preparation, or in some other solid form. The preparation dissolves very quickly in the stomach. The preparation is used to show increased urease activity in the stomach in association with an ongoing Helicobacter pylori infection.		

Helicobacter pylori. Urease catalyzes the degradation of urea to ammonia and bicarbonate. The bicarbonate is then converted to carbon dioxide and water in the acid environment of the stomach.

5 At present, the urea-breath test is performed in the following manner: The patient swallows an aqueous solution containing isotope-labelled (^{11}C , ^{13}C , ^{14}C) urea. In the presence of urease-producing *Helicobacter pylori* and acid in the stomach, urea is broken down so that the isotope-labelled carbon atoms convert to carbon dioxide and are secreted via the exhaled air. The amount of isotope-labelled carbon dioxide in the
10 exhaled air is determined.

The advantages of this method are that the examination is non-invasive, i.e. the patient need not be subjected to instrumentation (gastroscopy) and neither need blood samples be taken or samples of secretion taken from the stomach. Furthermore, because the
15 urea supplied to the patient disperses over the whole surface of the stomach, the fact that the bacterium has a very spotty distribution area lacks significance.

The urea-breath test is disadvantageous on three separate counts:

20 1. Isotope-labelled urea is ingested as an aqueous solution. This means that the urea can be broken down by urease-producing bacteria in the oral cavity and the pharynx of the patient, which may result in falsely positive indications regarding the presence of *Helicobacter pylori*.

25 2. In order for the test to be successful, it is necessary for a given quantity of hydrogen ions to be present in the stomach in order for the bicarbonate formed to be converted into carbon dioxide. Many patients who suffer from stomach ulcers are treated with an acid-suppression inhibitors which gives a falsely negative test result when such patients are diagnosed in accordance with present methodology.

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3. Isotope-labelled urea has only a short chemical durability in aqueous solution, which

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causes practical difficulties when the patient is tested clinically.

It will be evident from the foregoing that several diagnostic methods of showing the presence of *Helicobacter pylori* in the stomach are known to the art, but that these methods are encumbered with a number of disadvantages, for instance are deficient in positively showing the presence of bacteria or are unpleasant for the patient.

There is thus a need to improve such diagnostic methods so that a more reliable diagnosis can be made under all conditions, and to enhance the practical use of such methods.

General disclosure of the invention

By constructing a capsule, tablet or some other solid preparation that has a high rate of disintegration and contains isotope-labelled (^{11}C , ^{13}C , ^{14}C) urea in combination with an acid, the following four important advantages are obtained with urea-breath tests relating to the diagnosis of gastric ulcer diseases:

1. When isotope-labelled urea is administered in the form of a solid preparation, the degradation by urease-producing bacteria in the oral cavity and the pharynx before the urea has reached the stomach is prevented. This reduces the number of falsely positive results and therewith significantly increases the reliability of the examination.

2. The administration of a weak acid at the same time as the isotope-labelled urea is administered also enables the urea-breath test to be performed during ongoing acid-suppression inhibiting treatment. This is a significant gain, since the majority of the patients who are subjected to the urea-breath test are chronically treated with potent acid-secretion inhibiting drugs. This substantially reduces the occurrence of falsely negative results.

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3. Because isotope-labelled urea and a weak acid in capsule, tablet or other solid form,

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are stable over a relatively long period of time, the problems caused by the limited chemical stability of urea in aqueous solution are avoided.

This affords significant practical advantages with regard to distribution and application of the urea-breath test.

4. The use of a ready-prepared solid preparation (e.g. a tablet) also affords advantages with regard to dosage accuracy and time saving, in comparison with preparing the established water-based test liquid.

In order to be able to achieve these significant advantages, it is necessary to construct the solid preparation such that it affords protection of its contents from the influence of urease in the oral cavity and in the pharynx, and also such that it will break down rapidly and completely in the stomach.

Technical description of the invention

There is provided in accordance with the invention an essentially water-free preparation which contains primarily either ^{11}C , ^{13}C or ^{14}C labelled urea and an acid, for instance ascorbic acid, citric acid, tartaric acid or acigluamin, in a solid preparation intended for peroral administration to human beings. However, it should be noted that the invention is not restricted solely to urea and that the present invention also includes other substances or substrates that are influenced by the activity of *Helicobacter pylori* and that can be readily analyzed.

The preparation shall contain sufficient acid to produce a sufficiently low pH-value in the stomach when ingested. In this regard, a suitable pH value is beneath $\text{pH} = 4$. The amount of a specific acid required to obtain a desired pH in the stomach of a patient can be determined easily by the skilled person on the basis of simple routine tests.

The pharmaceutical preparation according to the invention is composed such that

isotope-labelled urea and acid will be released essentially immediately when the preparation reaches the stomach. A preferred form of the preparation includes ^{13}C urea and water-free citric acid. The preparation may either be a conventional tablet, optionally provided with a quickly soluble coating, although it may alternatively have the form of a capsule preparation. In this latter case, so-called hard gelatin capsules (operculate capsules) are intended, although soft gelatin capsules may also be used. The sole prerequisite is that the chemical stability is sufficiently high and that the active substance content of the preparation can be quickly released and dissolved. The chemical stability is normally improved when the active substances and auxiliary substances are present in solid form. The chemical stability will normally be sufficiently high when an oil solution is used in combination with a soft gelatin capsule.

Other solid preparations may also be used. Without limiting the list of those preparations that can be used in this regard, it can be mentioned that such preparations may include solid solutions, solid dispersions and so-called pellet preparations. By pellet preparations is meant larger or smaller grains produced by granulation or by extrusion/spheronization. The active substance can then be incorporated in these pellets or attached to their surfaces. The active pellets can then be compressed to a quickly disintegrating tablet form, or can be filled in hard gelatin capsules.

Tablets, which are the preferred form, can be produced with the aid of auxiliary substances and process steps well known to the skilled person. Thus, in order to obtain maximum rapid release and dissolution there are preferably used essentially water-soluble components and auxiliary substances which accelerate disintegration of the tablet. The auxiliary substances used may have the form of pharmaceutical bursting or disintegrating agents of a kind known to the skilled person. Particularly suitable agents in this regard are those which swell markedly in water by hydration, to a volume that corresponds to 10-20 times their dry volume. Examples of such agents include cellulose derivatives and starch derivatives in the form of cross-linked polymers which are insoluble in water but which swell markedly therein. Derivatives of polyvinyl pyrrolidone is another example of such agents. A modified cellulose-gum of high water

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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2020
Bogotá, D.C.

Abogado
/ **ALVARO CORREA ORDOÑEZ**

ASUNTO Radicación 06128821
 Trámite 002
 Evento 001
 Actuación 411
 Oficio 12504
 Folios 016

Patente de Invención ☒ Patente de Modelo de Utilidad ☐
Vía Nacional ☐
Vía PCT (fase nacional) ☒ Cap. I ☐ Cap. II ☒

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

- 1.1. Descripción: Folios: 62-78 como se radicó inicialmente
- 1.2. Reivindicaciones: Folios: 79-82 como se radicó inicialmente
- 1.3. Dibujos: Folios: 61 como se radicó inicialmente
- 1.4. Oposición: Folios 102 a 1164 de 03/04/ 2008.
 Sergio R. Cabrera Manriquez (Isomed S.L)
- 1.5. Prioridad: 18/12/2006; US; 11/611883

2. Objeto de la invención

Titulo de la invención: Método mediante una prueba de aliento para la detección de microorganismos patógenos

Problema Técnico Planteado: La necesidad de un kit para realizar la prueba de aliento. para la detección de microorganismos patógenos, que no requiere soluciones de ácido cítrico

Solución: un kit para realizar la prueba de aliento, para la detección de microorganismos patógenos, que no requiere soluciones de ácido cítrico y elimina la presencia de urea residual en la cavidad oral.

IPC: G01N33/497; G01N33/62; G01N33/60, C12Q11/58

3. De la solicitud de Patente

3.1. Descripción (artículo 28 D. 486)

No hay datos comparativos del kit solicitado frente al conocido (respecto al número de individuos positivos y negativos), porque en el folio 72, pg 11 se menciona que al usar el protocolo de referencia, 46 individuos fueron positivos y 24 negativos. Además, no se especifica cual protocolo corresponde al kit solicitado y cual, al del estado de la técnica. Por lo presentado en la tabla (I), se asume que el protocolo (I), es el del kit solicitado y el (II) el kit del estado de la técnica.

3.2. Reivindicaciones (artículo 30 D 486)

La reivindicación 13, esta caracterizada por el microorganismo *Helicobacter pylori*, el cual no hace parte de dicho kit.

4. Excepción a la patentabilidad (artículo 20 D486 literal d))

Las reivindicaciones 1-5, no son patentables de acuerdo a l presente artículo.

5. Análisis de la Oposición: (artículo 43 D. 486):

Oposición de Sergio R. Cabrera Manríquez (Isomed S.L) (folios 102-164)

Sergio R. Cabrera Manríquez, argumenta que la presente solicitud que consiste en un método y un kit para la detección de *Helicobacter pylori*, carece de Novedad y de Nivel Inventivo, frente al kit Taukit (folios 117-1209, pg16 de la respuesta).

Respuesta del examinador técnico a la oposición:

Esta oficina considera que el kit de la solicitud, es nuevo frente al patentado, porque incluye un recipiente con agua. No obstante no tiene nivel inventivo frente al ya divulgado, porque no se evidencia un efecto sorprendente logrado por esa diferencia. Por lo anterior se declara fundada la oposición.

Respuesta del solicitante: Alvaro Correa Ordóñez (Germán Campuzano (folios 165-253); de 23/07/ 2008):

A lo argumentado por el opositor (folio 167, pg3, respuesta a la oposición), el solicitante responde, que la solicitud cumple con el requisito de novedad porque: "En primer lugar el método divulgado en la patente N° 27386 involucra la administración de ácido cítrico al paciente como parte del método con lo cual se cumple con el requisito de novedad. De otra parte, el presente método, incorpora una etapa novedosa adicional al método desarrollado por Isomed S.L correspondiente a la administración oral de una cantidad adecuada de agua al paciente".

Se aclara primero: que los métodos de tratamiento no son patentables de acuerdo al *artículo 20 D486 literal d* y que respecto al kit reclamado, se realizo el presente análisis de patentabilidad y segundo que el kit reclamado es

diferente del divulgado, en que el primero contiene un recipiente con agua, por lo tanto se considera nuevo.

El solicitante, presenta un listado de diferencias entre el método de Isomed s.L y el método desarrollado por el Dr Campuzano, solicitante del kit aquí reclamado.

Se aclara que las diferencias ente los dos kit se basan en las características esenciales que definen a cada uno y no en resultados a alcanzar; no obstante, se hacen las siguientes aclaraciones con respecto a las diferencias citadas por el solicitante:

Prueba Colombiana Solicitud	Tau-Kit de Isomed S.L (D1)	Respuesta del examinador técnico a la respuesta del solicitante, frente a la oposición:
Utiliza baja dosis de urea marcada con C ¹³ 50mg	Utiliza alta dosis de urea marcada con C ¹³ 100mg	La dosis de urea de D1 es 0.15%(reiv 12) y la del Kit reclamado es 0,33%, es más baja la de D1.
No utiliza ácido cítrico	Utiliza 3,2g de ácido cítrico	La reiv 6 de la solicitud dice, kit que comprende; de esta manera, puede comprender otro elemento como el ácido cítrico de D1; además en D1, se menciona que la ureasa producida por el Helicobacter Pylori, se degradada a bicarbonato y este a su vez a CO2 (que se recoge en el segundo recipiente para analizar y determinar la presencia del microorganismo) y agua en el medio ácido del estómago, lo cual puede sugerir la no presencia de este ácido (folio 260, pg3). D1, también menciona cual es el objetivo de la presencia del ácido cítrico en el Kit, divulgado (folio 255, columna 4, líneas 5-9), y no se observa una ventaja por la no presencia de este ácido en el kit reclamado.
Riesgo para el paciente: absolutamente inocua	Riesgo para el paciente: Nauseas y vómito, sobretudo en niños y aumento de la hiperacidez gástrica en pacientes con síntomas de dispepsia y úlceras.	A pesar de que no es una característica esencial del kit, ya D1, mencionaba las ventajas del mismo (folio 256, líneas 20-23).
No altera la fisiología gástrica		A pesar de que no es una característica esencial del kit, ya D1, mencionaba las ventajas del mismo (folio 256, líneas 20-23).
Con la forma de administrarla, como "un trago fuerte" que se "pasa rápidamente" con 200ml. de agua se eliminan los falsos positivos relacionados con bacterias productoras de ureasa en la boca y en la orofaringe.	Falsos positivos por bacterias productoras de ureasa en la boca y en la orofaringe.	A pesar de que no es una característica esencial del kit, ya, en el estado del arte D2 WO9614091, advertía la posibilidad de falsos positivos (folio 260, líneas 20-23).
Estimula el contacto de la urea marcada con C13 con la totalidad de la mucosa gástrica...en la solución acuosa que se forma en el estómago.	Falsos negativos por mala distribución de la urea en la mucosa gástrica.	A pesar de que no es una característica esencial del kit, ya, en el estado del arte D2 WO9614091, advertía que reduce los falsos negativos (folio 261, líneas 28-29).
Es corta (10 minutos)	Es larga (30 minutos)	A pesar de que no es una característica esencial del kit, en la descripción de la solicitud (folio 73) se muestra que ambas pruebas tienen una duración de 30 minutos.
Sensibilidad 100%	Sensibilidad 94,3%	A pesar de que no es una característica esencial del kit, en la tabla (I), folio 73, pg 12, se muestra una sensibilidad de 97, 83%(folio 74, pg 13), y no se evidencia a que característica esencial diferente del kit divulgado, se debe esa pequeñísima diferencia.
Especificidad 100%	Especificidad 96,3%	A pesar de que no es una característica esencial del kit, en el folio 74, el solicitante concluye que la sensibilidad del método conocido es de 97.83% frente al 100% con el kit solicitado, lo cual no muestra que el kit reivindicado dé un efecto sorprendente.
Utiliza dos tubos	Utiliza 4 tubos	D1, menciona al menos 2 recipientes destinados a contener el aliento del paciente (folio 256, columna 8, líneas 44-45)
Es barata, lo cual permite masificarla	Es cara, lo cual restringe su masificación	A pesar de que no es una característica esencial del kit, no se muestran costos, no era el objeto de la invención, y el Kit de D1 se utiliza ampliamente en humanos.

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Esta oficina aclara que, aunque el solicitante menciona argumentos mediante los cuales considera que su solicitud (kit) si es nueva, frente al kit, Taukit, ya patentado, y por esa razón no es fundada la oposición; la presente solicitud (kit) carece de nivel inventivo frente al kit Taukit (ES2120903). Así mismo, se aclara que dado que el kit de la reivindicación 6 es general, puede contener otras cosas, como el ácido cítrico, que se menciona en D1.

El solicitante argumenta que su solicitud tiene nivel inventivo porque al no contener ácido cítrico se evitarían efectos adversos.

Se aclara que la diferencia con el kit de D1, es que el reclamado menciona específicamente que contiene un recipiente con agua, y que la no presencia del ácido cítrico no muestra una ventaja sobre el kit divulgado (aclaración de la tabla de diferencias citada arriba por el solicitante). Además, como no se evidencia un efecto logrado por contener un recipiente con agua, dado que igualmente se logra, la detección del *Helicobacter Pylori*, con la misma especificidad, confianza y la misma alta probabilidad para clasificar individuos infectados y cero probabilidad para clasificar individuos no infectados, se concluye que el kit reivindicado no presenta nivel inventivo.

El solicitante presenta copias de un artículo(folio 185-195) sobre la aplicación del kit reclamado, en donde menciona que es una optimización del test de aliento con urea marcada con C¹³ para diagnóstico del Helicobacter Pylori (folio 172, pg 8); de una prueba que se conoce desde 1924 (folio 170); es importante aclarar que para el experto en la materia es de su rutina, realizar optimizaciones o adaptaciones lo cual le permitió obtener el kit reivindicado sin que esto le confiera altura inventiva a dicho kit.

6. Determinación del Estado de la Técnica

La fecha que se ha tenido en cuenta para determinar el Estado de la Técnica es:

Fecha de presentación de la solicitud prioritaria 18/12/2006.

Teniendo en cuenta la fecha indicada se realizó la búsqueda en el estado de la técnica de documentos relacionados con la solicitud encontrándose los siguientes documentos más relevantes:

Nº	Documento	Título	Fecha de publicación*
D1	ES2120903	Método y Kit para la detección de Helycobacter pylori	01/11/1998
D2	WO9614091	Diagnostic preparation for detection of Helicobcter pylori	05/17/1996

D1 (folios: 254-258); D2 (anterioridad tenida en cuenta para el estudio de la patente con certificado N° 27386)

7. Análisis de patentabilidad (artículo 14 D486)

7.1 Nivel inventivo (artículo 18 D486)

La reivindicación 6 consiste en:

Un kit para la detección de microorganismos patógenos mediante pruebas de aliento, en donde el kit que comprende:

- a) Un recipiente con agua
- b) Un recipiente con urea con carbón marcado
- c) Un primer receptáculo para recoger una primera muestra de aliento al principio de la prueba de aliento y antes de la ingestión de urea con carbón marcado.
- d) Un segundo receptáculo para recoger una segunda muestra de aliento y después de la ingestión de una cantidad adecuada de agua; y en donde el primer receptáculo y el segundo receptáculo tienen tapas con un mecanismo que permiten la introducción de un sensor en forma de agujas.

El Estado de la Técnica más cercano es D1 (ES2120903) que da a conocer un kit (folio 256, columna 8) para la detección de *Helicobacter Pylori*, que comprende un estuche que contiene:

Un recipiente que contiene urea marcada con carbono 13.

Al menos, dos recipientes destinados a contener el aliento del paciente, uno de los cuales esta destinado a contener el aliento del paciente tras la administración del ácido cítrico pero antes de la administración de la urea marcada con carbono 13, mientras que el otro recipiente está destinado a contener el aliento del paciente tras la administración de la urea marcada con carbono -13, estando dichos recipientes constituidos por unos tubos de cristal provistos de unos tapones que van a roscas y tienen un septum; y

Unos medios para soplar el aliento dentro de los recipientes antes citados.

El kit objeto de esta solicitud se diferencia del de D1, en que el solicitado contiene un recipiente con agua.

No se evidencia un efecto técnico logrado, cuando el kit solicitado contiene un recipiente con agua; ya que los resultados de las pruebas aplicadas mediante el kit de referencia y el reclamado, le permiten al solicitante llegar a las siguientes conclusiones (folio 74, pg13): con ambos kit se obtiene la misma especificidad, la misma confianza del 95%, la misma alta probabilidad para clasificar individuos infectados y cero probabilidad para clasificar individuos no infectados; además el kit reivindicado sirve para lo mismo (detección de microorganismos patógenos como el *Helicobacter Pylori*) que el kit del estado del arte. Además, D1 (folio 258, columna 149, reivindicación 12), ya mencionaba la cantidad de urea entre 75 y 100mg en 20 a 50 mL de agua, (al 0.15%), que comparada con la cantidad de urea marcada en el kit reclamado, 50mg en 15mL (al 0.33%), se observa que tampoco hay ventaja sobre el kit divulgado.

El Problema Técnico objetivo era "cómo obtener un kit, para la detección de microorganismos patógenos, *Helicobacter Pylori*, mediante pruebas de aliento, alternativo, al ya divulgado en D1".

Por tanto, la solicitud, tal como se reivindica, es obvia, porque el Experto en la Materia enfrentado al problema de "cómo obtener un kit, para la detección de microorganismos patógenos mediante pruebas de aliento, alternativo, al divulgado en D1", que contenga un recipiente con agua (200mL) incluiría los 200mL de agua (D1, folio 258, columna 14, reiv 11), logrando de esta forma el kit objeto de esta solicitud; sin que la presente reivindicación presente altura inventiva, como tampoco sus dependientes, 7-12.

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8. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

N°	Documento N°	Reivindicaciones afectadas	Requisito que afecta
D1	ES2120903	6-12	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.

ALIX CARMENZA CÉSPEDES DE VERGEL
Dirección de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha
23 SET 2010

CONCEPTO TÉCNICO No. 3078	EXAMINADOR TÉCNICO Código No.202078
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