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Papers

Diagnosis of *Helicobacter pylori* infection by specific gastric mucosal IgA and IgG pylori antibodies

R A Veenendaal, J M Götz, V Schroijen, F Kurban, A T Bernards, M Veselic, A S Peña, C B H W Lamers

Abstract

***Aims*—To investigate the diagnostic value of mucosal IgA and IgG *Helicobacter pylori* antibodies.**

***Methods*—The study population comprised 209 consecutive patients with severe dyspeptic complaints referred for upper gastrointestinal endoscopy. A positive culture or histological identification of *H pylori* in gastric biopsy specimens, or both, were used to confirm infection. Specific IgA and IgG *H pylori* antibodies were determined using a modified ELISA technique.**

***Results*—Of the 209 patients, 137 were infected with *H pylori*. The diagnostic value of systemic IgA and IgG *H pylori* antibodies was confirmed. Systemic IgA antibodies had a sensitivity of 76·6% (95% confidence interval 69·5–83·7) and a specificity of 94·4% (89·1–99·7). The sensitivity and specificity for systemic IgG antibodies were, respectively, 97·1% (94·3–99·9) and 98·6% (95·9–100). A moderate but clinically important correlation was found between local and systemic IgA and IgG. Mucosal IgA *H pylori* antibodies had a sensitivity of 98·5% (96·5–100) and a specificity of 91·7% (85·3–98·1), while for IgG these figures were, respectively, 88·3% (82·9–93·7) and 98·6% (95·9–100). As a diagnostic test mucosal IgA *H pylori* antibodies were comparable with culture and histology.**

***Conclusion*—Determination of local IgA and IgG *H pylori* antibody levels is a highly sensitive and specific test for the diagnosis of *H pylori* infection.**

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Keywords: ELISA, *Helicobacter pylori*, IgA, IgG, serology.

Department of Gastroenterology and Hepatology, University Hospital Leiden, Building 1, C4-P, PO Box 9600, 2300 RC Leiden, The Netherlands
R A Veenendaal
J M Götz
V Schroijen
F Kurban
C B H W Lamers

Department of Medical Microbiology
A T Bernards

Department of Pathology, Leiden University Hospital
M Veselic

Department of Gastroenterology, Free University Hospital, Amsterdam
A S Peña

Correspondence to:
Dr R A Veenendaal.

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Ten years after the successful culture of *Helicobacter pylori*,[1 2] major progress has been made in the understanding of the pathogenic importance of this bacterium. *H pylori* causes chronic active gastritis,[3 4] is one of the most important pathogenic factors in peptic ulcer disease[5 6] and may have a role in the pathogenesis of gastric carcinoma.[7-9]

Several tests for diagnosing *H pylori* infection have been developed. These can be divided into direct tests, for which gastric biopsy specimens are required, and indirect tests which rely on serum or breath ($^{13}CO_2$, $^{14}CO_2$) samples. Investigators are still polarised as to which test should be used for the diagnosis of *H pylori* infection. Proponents of indirect testing argue that upper gastrointestinal endoscopy is unnecessary and expensive, while for those in favour of direct tests the opportunity to detect macroscopic disease during endoscopy is paramount.[10] In the present study we examined the diagnostic value of detecting local (direct) and systemic (indirect) IgA and IgG antibodies against *H pylori* using a modified in-house enzyme linked immunosorbent assay (ELISA) and compared this with culture and histology.

Methods

The study population comprised 209 consecutive patients (120 men and 89 women) with severe dyspeptic complaints who were referred for upper gastrointestinal endoscopy. Diagnostic tests for *H pylori* related gastritis were requested by the referring physician in all cases. Patients with an upper gastrointestinal malignancy and those taking antibiotics, bismuth preparations or omeprazole in the three months prior to the endoscopy were excluded.

After an overnight fast, an upper gastrointestinal endoscopy was performed in all patients and blood was obtained for serological tests. At endoscopy, biopsy specimens were taken from intact mucosa in the antrum 3–5 cm proximal to the pylorus. One biopsy specimen was set aside for culture of *H pylori*[11] and was transported to the laboratory within two hours in a small sterile glass jar with a screw-cap in 2 ml sterile 0·9% NaCl. The biopsy specimen was cultured on blood agar (Blood Agar Base No 2, Oxoid CM 271, containing 5% sheep's blood) and Skirrow's medium, and cultures were incubated at 37°C in a nitrogen atmosphere containing 8% CO_2 and 6% O_2 for five days. The bacteria were identified as *H pylori* on the basis of their morphology, and oxidase, catalase, and urease production.

Four biopsy specimens, two from the anterior and two from the posterior wall, were obtained for histological assessment and measurement of local IgA and IgG *H pylori* antibodies. The

two specimens for histological examination were immediately fixed in 10% buffered formalin, embedded in paraffin wax and cut into 4 μm thick serial sections, which were stained with haematoxylin and eosin. The histological detection of *Helicobacter*-like organisms was based on the identification, by an experienced pathologist, of micro-organisms with appropriate morphology, location, and staining characteristics in the mucosal biopsy specimens. Additional staining techniques (Giemsa, Whartin–Starry) were used where necessary.

Specific IgA and IgG antibodies against *H pylori* were measured in serum and in snap frozen antral biopsy specimens using a modified ELISA.

Antigen was prepared for the ELISA as follows: a mixture of six pooled *H pylori* strains (whole bacteria) was sonicated for six minutes. The suspension was adjusted to a protein concentration of 3 mg/ml. The optimum concentration of reagents was determined by checker board titration as described elsewhere.[12]

Each well of a flat bottom polystyrene microtitre plate (Dynatech Laboratories, Chantilly, Virginia, USA; M129A) was coated with 100 μl antigen solution (10 μg suspension/ml carbonate buffer, pH 9·6, for IgA and the local IgA and IgG antibodies, and 1 μg suspension/ml for systemic IgG) and incubated overnight at room temperature. The plates were washed three times with phosphate buffered saline (PBS) (pH 7·5) containing 0·05% Tween 20.

Systemic specific IgA and IgG antibodies directed against *H pylori* were measured in serum, diluted 1 in 200 in PBS/Tween 20, by a modified ELISA technique using conjugates labelled with immunoperoxidase specific for human IgA and IgG. The following conditions were used to standardise antibody measurement: the mean (SD) values for absorbance of the standard reference serum were 0·5 (0·1) for IgA and 1·0 (0·1) for IgG. These values were used to correct the absorbance given by the serum samples under study.

The absorbance index (AI) was calculated from the mean of two readings of the optical density (OD) of the serum. The results were expressed as follows:

$$\text{AI} = \frac{\text{Patient's OD} - \text{OD of blank reading}}{\text{Reference OD} - \text{OD of blank reading}}$$

Intra- and interassay variabilities were determined as described in detail elsewhere.[12]

Local specific IgA and IgG antibodies directed against *H pylori* were also measured using a modified ELISA technique.

Biopsy samples were weighed and homogenised (Braun Potter S) in 300 μl PBS/Tween 20 and then adjusted to 1 mg/100 μl and stored at −20°C. The samples were then diluted 1 in 100 in PBS/Tween 20 and 100 μl was added to each well. Two reference samples (one positive and one negative) were also assayed. The plates were incubated at room temperature for 90 minutes and then washed three times in PBS/Tween 20. Diluted goat anti-human peroxidase (100 μl) was added to each well (Pasteur Institute, Paris, France; code no. 75051 diluted 1:1000 in PBS/Tween 20 for IgG and code no. 75041 diluted 1 in 2500 in PBS/Tween 20 for IgA). The plates were then incubated for 90 minutes at room temperature, washed and 100 μl substrate (0·40 mg/ml O-phenylenediaminedihydrochloride (Sigma, St Louis, Missouri, USA) containing 0·012% H_2O_2) was added. After 30 minutes the reaction was stopped with 50 μl 2·5 M H_2SO_4. The OD was measured at 492 nm on the Titertek Multiscan plate reader (Flow Laboratories, Irvine, Scotland, UK).

Before the data were analysed, the protein concentration (Lowry) of each biopsy homogenate was determined. To standardise the measurement of these antibodies, one positive serum (diluted 1 in 500) was tested more than 10 times in duplicate and the mean OD was used as a standard reference.

The results were expressed as follows:

$$\text{AI} = \text{Mean OD reading (n=2) of biopsy homogenate} - \text{mean OD of blank reading} \times \frac{\text{mean OD reading (n=2) of reference} - \text{mean OD of blank reading}}{\text{mean OD reading (n=10) of reference}} \times \frac{10\ (\%\ \text{protein})}{\text{protein conc. of the homogenate (\%)}}$$

Intra-assay variability for specific IgA *H pylori* antibodies was determined by measuring 17 homogenates (five with a low titre, six with a moderate titre and six with a high titre) six times in one plate, which resulted in a coefficient of variation of 6·9%. Intra-assay variability for specific IgG *H pylori* antibodies was also determined by measuring 17 homogenates (nine with a low titre, four with a moderate titre and four with a high titre) six times in one plate, which resulted in a coefficient of variation of 10·0%.

Interassay variability was determined by measuring specific IgA and IgG *H pylori* antibodies in the same biopsy homogenates (in duplicate) over five different days which resulted in coefficients of variation of 12·1% and 15·9%, respectively.

STATISTICAL ANALYSIS

The significance of differences between test results in *H pylori* positive and negative patients was assessed using the Student's *t* test. To correct for multiplicity a p value <0·01 was chosen as the criterion for statistical significance. The significance of differences between the single diagnostic tests used was assessed using the χ^2 test.

Results

Of the 209 patients included in the study, 72 (mean age (SD) 47 (15) years) were *H pylori* negative while 137 (mean age (SD) 51 (13) years) were positive for *H pylori* on culture and/or histology. The diagnostic findings at upper gastrointestinal endoscopy are summarised in table 1.

In *H pylori* positive patients specific local (mean (SEM) 140·3 (5·2) *v* 2·8 (0·5), p<0·01 and systemic (mean (SEM) 81·6 (4·5) *v* 21·2

Table 1 Diagnoses at upper gastrointestinal endoscopy in H pylori *negative and* H pylori *positive patients with severe dyspeptic complaints*

	Patients	
Diagnosis at endoscopy	H pylori *positive* *(n=137)*	H pylori *negative* *(n=72)*
No abnormalities	45 (32·8%)	43 (59·7%)
Duodenal ulcer	48 (35·0%)	1* (1·4%)
Gastric ulcer	15 (10·9%)	2 (2·8%)
Reflux oesophagitis	13 (9·5%)	17 (23·6%)
Other diagnosis	16 (11·7%)	9 (12·5%)

* Associated with non-steroidal anti-inflammatory drug use.

Table 2 Differences between local and systemic specific IgA and IgG H pylori *antibodies in* H pylori *positive and* H pylori *negative patients. Results given as mean (SEM)*

	Patients		
H pylori *antibodies* *(AI x 100)*	H pylori *positive* *(n=137)*	H pylori *negative* *(n=72)*	*p value**
Local IgA	140·3 (5·2)	2·8 (0·5)	p<0·01
Systemic IgA	81·6 (4·5)	21·2 (3·3)	p<0·01
Local IgG	32·3 (2·5)	1·6 (0·3)	p<0·01
Systemic IgG	76·2 (1·8)	13·9 (0·9)	p<0·01

* Student's *t* test.

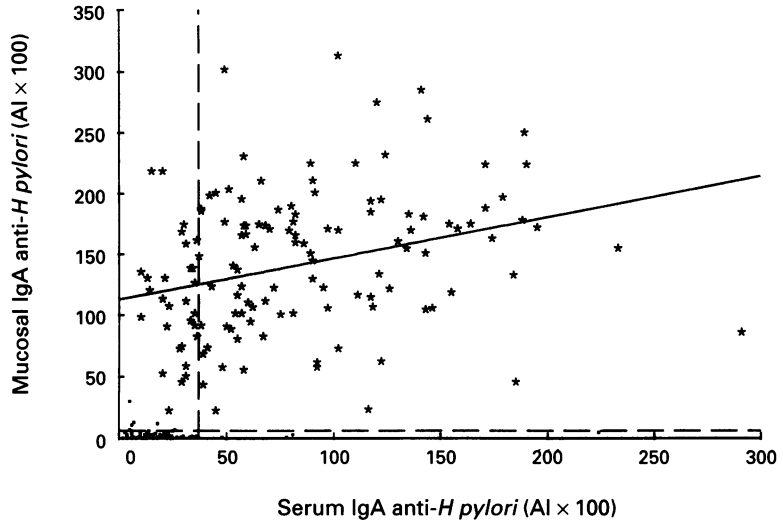

Figure 1 Mucosal IgA H pylori *antibodies versus systemic IgA* H pylori *antibodies in* H pylori *positive (★) and negative (●) patients. Line: regression (r=029; p<0·01) line in* H pylori *positive patients. The dotted lines indicate the cut off levels used in both assays.*

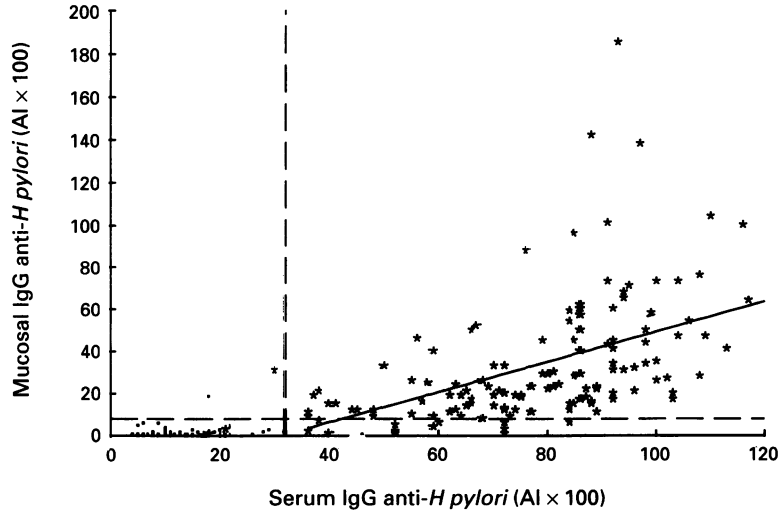

Figure 2 Mucosal IgG H pylori *antibodies versus systemic IgG* H pylori *antibodies in* H pylori *positive (★) and negative (●) patients. Line: regression line (r=052; p<0·01) in* H pylori *positive patients. The dotted lines indicate the cut off levels used in both assays.*

(3·3), p<0·01) IgA *H pylori* antibody levels (AI x 100) were significantly higher than those in *H pylori* negative patients (table 2). Specific local (mean (SEM) 32·3 (2·5) *v* 1·6 (0·3), p<0·01) and systemic (mean (SEM) 76·2 (1·8) *v* 13·9 (0·9), p<0·01) IgG *H pylori* antibody levels (AI x 100) were also significantly higher in *H pylori* positive patients (table 2). A weak but significant correlation (r=0·29, p<0·01) between local and systemic IgA *H pylori* antibody levels was found in *H pylori* positive patients (fig 1). A moderate but significant correlation between local and systemic antibody levels (r=0·52, p<0·01) was found for IgG *H pylori* antibodies (fig 2).

The normal range, for both systemic and local IgA and IgG *H pylori* antibodies, was defined as the 95% confidence interval of the mean absorbance indices (AI) in 49 *H pylori* negative patients without histological evidence of gastritis. Under the conditions of the ELISA technique described, local IgA *H pylori* antibodies with an AI>0·06 and systemic IgA *H pylori* antibodies with an AI>0·37 indicated a positive *H pylori* infection. Local IgG *H pylori* antibodies with an AI>0·08 and systemic IgG *H pylori* antibodies with an AI>0·32 were also regarded as indicative of a positive *H pylori* infection. Using these criteria, determination of mucosal IgA *H pylori* antibody levels falsely classified six patients as *H pylori* positive and two as *H pylori* negative. Systemic IgA *H pylori* antibodies falsely classified four patients as *H pylori* positive and 32 as *H pylori* negative. These findings resulted in a sensitivity and specificity of, respectively, 98·5% (95% confidence interval 96·5–100) and 91·7% (85·3–98·1) for mucosal IgA *H pylori* antibodies and, respectively, of 76·6% (69·5–83·7) and 94·4% (89·1–99·7) for systemic IgA *H pylori* antibodies. Mucosal IgG *H pylori* antibodies falsely classified one patient as *H pylori* positive and 16 as *H pylori* negative. Systemic IgG *H pylori* antibodies falsely classified one patient as *H pylori* positive and four as *H pylori* negative. These findings resulted in a sensitivity and specificity of, respectively, 88·3% (82·9–93·7) and 98·6% (95·9–100) for mucosal IgG *H pylori* antibodies and, respectively, of 97·1% 94·3–99·9) and 98·6% (95·9–100) for systemic IgG *H pylori* antibodies.

The results of each individual test, with regard to the *H pylori* status of the patients, are shown in table 3 and compared with results of culture, histology, and culture and histology. There was no significant difference (p=0·13) among gastric mucosal IgA antibodies, culture, histology, and culture and histology for the diagnosis of *H pylori* infection.

Discussion

Helicobacter pylori induces a specific local[13–15] and systemic[12 16 17] immune response with the development of mucosa associated lymphoid tissue,[14 18 19] which is absent in normal stomach. The inflammatory changes in the gastric mucosa associated with this specific immune response are described as active chronic or chronic gastritis depending on the presence or

Table 3 Results of the different diagnostic tests performed in this study for determination of the H pylori *status of the patients in relation to culture or histology, or both.*

Diagnostic tests for H pylori	*Positive tests*	*Negative tests*
Systemic IgA	109	100
Systemic IgG	134	75
Local IgA	141	68
Local IgG	122	87
Culture	121	88
Histology	125	84
Culture and/or histology*	137	72

* $p = 0{\cdot}13$, χ^2 test.

absence of leucocytes and lymphocytes infiltrating the gastric mucosa.[20] These inflammatory changes are probably directly or indirectly responsible for functional derangements in the gastric physiology and mucosal defence mechanisms leading to peptic ulcer disease. Although the specific humoral immune response in most patients apparently does not lead to elimination of the bacterium from the gastric mucosa,[21] it has successfully been used to diagnose *H pylori* infection serologically using systemic circulating specific IgA and IgG *H pylori* antibodies.[12 16] Besides serological detection of *H pylori* infection *H pylori* antibodies have also successfully been used for follow up[22-24] and in epidemiological studies.[8 9 25] In the present study we confirmed the high sensitivity (97·1%) and specificity (98·6%) of systemic IgG *H pylori* antibodies which correlated significantly with mucosal IgG antibodies. Although systemic IgA *H pylori* antibodies were highly specific (94·4%), they were less sensitive for detecting *H pylori* infection (76·6%), indicating that the predominant local production of IgA does not lead to high systemic IgA antibody levels in all patients (fig 1) rendering this test less suitable for diagnostic or epidemiological studies.

When compared with other more generally used non-invasive tests for diagnosing *H pylori* infection only the urea breath test[26 27] has a sensitivity and specificity comparable with that of the IgG ELISA. The applicability of the breath tests is, however, hampered by its requirement for equipment and its cost.[28] With a growing number of commercial kits for IgA and IgG *H pylori* antibodies becoming available it seems most likely that this non-invasive test will be more generally used. Direct tests can have low sensitivity[10] and high inter- and intra-observer variation especially when the number of bacteria is reduced by suppressive treatment with bismuth preparations, antibiotics, or proton pump inhibitors.[29] The direct test described herein may also faciliate follow up by demonstrating a decrease in the local production of *H pylori* antibodies. For oral vaccines against *H pylori* to be successful, the induction of a specific local IgA immune response is necessary. The level of local IgA *H pylori* antibodies necessary for preventing reinfection (after treatment) or conveying protection (after oral vaccination) has yet to be established. In conclusion, determination of gastric mucosal IgA and IgG *H pylori* antibody levels is highly sensitive and specific for the diagnosis of *H pylori* infection.

1 Warren JR. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;**i**:1273.
2 Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;**i**:1273-4.
3 Dooley CP, Cohen H, Fitzgibbons PL, Bauer M, Appleman MD, Perez-Perez GI, *et al.* Prevalence of Helicobacter pylori infection and histologic gastritis in asymptomatic persons. *N Engl J Med* 1989;**321**:1562–6.
4 Blaser MJ. Helicobacter pylori and the pathogenesis of gastroduodenal inflammation. *J Infect Dis* 1990;**161**:626–33.
5 Graham DY, Lew GM, Klein PD, Evans DG, Evans DJ, Saeed ZA, *et al.* Effect of treatment of Helicobacter pylori infection on the long-term recurrence of gastric or duodenal ulcer. *Ann Intern Med* 1992;**116**:705–8.
6 Axon AR. Duodenal ulcer: The villain unmasked? Eradicating Helicobacter pylori will cure most patients. *BMJ* 1991;**302**:919–21.
7 Graham DY. Treatment of peptic ulcers caused by Helicobacter pylori. *N Engl J Med* 1993;**328**:349–50.
8 Forman D, Newell DG, Fullerton F, Yarnell JWG, Stacey AR, Wald N, *et al.* Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. *BMJ* 1991;**302**:1302–5.
9 Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, *et al.* Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991;**325**:1127–31.
10 Barthel JS, Everett DE. Diagnosis of Campylobacter pylori infections: The "Gold Standard" and the alternatives. *Rev Infect Dis* 1990;**12**:107–14.
11 Veenendaal RA, Lichtendahl-Bernards AT, Peña AS, Endtz HPh, van Duijn W, van Boven CPA, *et al.* Influence of transport medium and transportation time on culture of Helicobacter pylori from gastric biopsies. *J Clin Pathol* 1993;**46**:561–3.
12 Peña AS, Endtz HPh, Offerhaus GJA, Hoogenboom-Verdegaal A, van Duijn W, de Vargas N, *et al.* Value of serology (ELISA and immunoblotting) for the diagnosis of Campylobacter pylori infection. *Digestion* 1989;**44**:131–41.
13 Rathbone BJ, Wyatt JI, Worsley BW, Shires SE, Trejdosiewicz LK, Heatley RV, *et al.* Systemic and local antibody responses to gastric Campylobacter pyloridis in non-ulcer dyspepsia. *Gut* 1986;**27**:642–7.
14 Wyatt JI, Rathbone BJ. Immune response of the gastric mucosa to Campylobacter pylori. *Scand J Gastroenterol* 1988;**23**(Suppl 142):44–9.
15 Peña AS, Veenendaal RA, Hohmann K, Dieleman J, Endtz HPh, Kreuning J, *et al.* Local antibody response to Helicobacter pylori infection. In: *Abstracts of the world congresses of gastroenterology.* Abingdon: The Medicine Group, 1990: 576.
16 Perez-Perez GI, Dworkin BM, Chodos JE, Blaser MJ. Campylobacter pylori antibodies in humans. *Ann Intern Med* 1988;**109**:11–17.
17 Evans DJ, Evans DG, Graham DY, Klein PD. A sensitive and specific serologic test for detection of Campylobacter pylori infection. *Gastroenterology* 1989;**96**:1004–8.
18 Stolte M, Eidt S. Lymphoid follicles in antral mucosa: immune response to Campylobacter pylori? *J Clin Pathol* 1989;**42**:1269–71.
19 Genta RM, Hammer HW, Graham DY. Gastric lymphoid follicles in Helicobacter pylori infection: frequency, distribution, and response to triple therapy. *Hum Pathol* 1993; **24**:577–83.
20 Price AB. The sydney system: histological division. *J Gastroenterol Hepatol* 1991;**6**:209–22.
21 Kuipers EJ, Peña AS, van Kamp A, Uyterlinde AM, Pals A, Pels NFM, *et al.* Seroconversion for Helicobacter pylori. *Lancet* 1993;**342**:328–1.
22 Veenendaal RA, Peña AS, Meijer JL, Endtz HPh, van der Est MMC, van Duijn W, *et al.* Long-term serological surveillance after treatment of Helicobacter pylori infection. *Gut* 1991;**32**:1291–4.
23 Oderda G, Vaira D, Holton J, Osborn J, Altare F, Ansaldi N. Eighteen month follow up of Helicobacter pylori positive children treated with amoxycillin and tinidazole. *Gut* 1992; **33**:1328–30.
24 Kosunen TU, Seppälä K, Sarna S, Sipponen P. Diagnostic value of decreasing IgG, IgA and IgM antibody titres after eradication of Helicobacter pylori. *Lancet* 1992;**339**: 893–5.
25 Sierra R, Muñoz N, Peña AS, Biemond I, van Duijn W, Lamers CBHW, *et al.* Antibodies to Helicobacter pylori and pepsinogen levels in children from Costa Rica: comparison of two areas with different risks for stomach cancer. *Cancer Epidemiology, Biomarkers and Prevention* 1992;**1**: 449–54.
26 Marshall BJ, Surveyor I. Carbon-14 urea breath test for the diagnosis of Campylobacter pylori associated gastritis. *J Nucl Med* 1988;**29**:11–16.
27 Graham DY, Klein PD, Evans DJ, Evans DG, Alpert LC, Opekun AR, *et al.* Campylobacter pyloridis detected non invasively by the ^{13}C-urea breath test. *Lancet* 1987;**i**:1174–7.
28 Berstad K, Berstad A. Helicobacter pylori infection in peptic ulcer disease. *Scand J Gastroenterol* 1993;**28**:561–7.
29 Christensen AH, Ajørup T, Hilden J, Fenger C, Henriksen B, *et al.* Observer homogeneity in the histologic diagnosis of Helicobacter pylori. Latent class analysis, Kappa coefficient, and repeat frequency. *Scand J Gastroenterol* 1992; **27**:933–9.