Accuracy of serology for the diagnosis of *Helicobacter pylori* infection—a comparison of eight kits

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Abstract

***Aims*—To determine the accuracy of eight commercially available kits for the serological diagnosis of *Helicobacter pylori* infection, and hence whether a serology service could be introduced to reduce endoscopy workload.**

***Methods*—Eighty four patients newly presenting to their general practitioners with dyspepsia were recruited. Gold standard diagnosis of *H pylori* infection was obtained both by a histological examination of gastroduodenal biopsy specimens and by the ^{14}C-urea breath test (UBT). The performance of six quantitative and two qualitative enzyme linked immunosorbent assays for *H pylori* IgG, used according to the manufacturers' instructions, with serum samples obtained during the endoscopy visit, were compared.**

***Results*—The study population had a median age of 45 years, and the prevalence of *H pylori* infection was 35%. With one exception, where the patient had received a course of anti-*H pylori* treatment between endoscopy and UBT, there was 100% concordance in the results of the two gold standard techniques. Discordant serology results were more common in patients aged >50 years (42% of the total) than in younger patients (21%), and this was most noticeable in uninfected patients. The sensitivity of the kits was good (90–100%), but specificity was more variable (76–96%), and the rate of equivocal results was unacceptably high in some cases (0–12%). The overall accuracy of the kits ranged from 83 to 98%. Two kits in particular performed well (Pylori-Elisa II, Bio-Whitaker and Premier, Launch; qualitative) with 98% and 100% accuracy, respectively.**

***Conclusions*—In a symptomatic population with a prevalence of *H pylori* infection of 35%, particularly in patients aged <50 years, some but not all serology kits may be used as a highly accurate and inexpensive alternative to the gold standard techniques.**

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Helicobacter pylori is now established as a cause of gastritis, and of gastric and duodenal ulcers. Recent consensus guidelines in the USA have advocated that patients with *H pylori* infection are treated with both antisecretory drugs and antibiotics.[1] Furthermore, *H pylori* has now been classified as a Group 1 (definite) carcinogen by the World Health Organisation, because of the evidence for its role in the pathogenesis of gut mucosa associated lymphoma and possibly also in gastric carcinoma. Currently, two gold standard techniques exist for the detection of *H pylori* infection. Upper gastrointestinal endoscopy with either culture or histological examination of biopsy tissue is highly specific, but in some cases problems of sensitivity relating to sampling technique have been experienced.[2] Nevertheless, endoscopy based techniques for the detection of *H pylori* have become widely accepted.[3] Urea breath testing, using either ^{13}C- or ^{14}C-urea, effectively samples the entire stomach, and improved sensitivity compared with endoscopy has been reported.[4] There is now a great demand for diagnostic services to detect *H pylori* infection and an alternative approach is required to reduce the increasing workloads of endoscopy and nuclear medicine units.

Serology has many attractions for the diagnosis of *H pylori* infection. It is inexpensive, essentially non-invasive, quick and easy to perform requiring little specialised equipment, and does not rely on the accuracy of the sampling technique to detect infection. Although many serology kits for the diagnosis of *H pylori* infection are now available commercially, including new generation kits, few UK microbiology laboratories are presently offering serology based diagnostic services.[5] A recent survey found that 75% of laboratories have no on-site *H pylori* serology service, but of these 71% (approximately half of the total) refer serum samples elsewhere.[5] One of the reasons for the slow uptake of serology to diagnose *H pylori* infection is uncertainty about the accuracy of currently available kits. Few studies have compared a large number of kits, and recent reports have not included both gold standard techniques for the detection of *H pylori* infection.[6–8] Consequently, they may have resulted in artefactually low specificities. The positive and negative predictive values of a test result are dependent on the prevalence of the condition in the sample population. We wished, therefore, to determine the local prevalence of infection with *H pylori* in patients presenting with dyspepsia, and to assess the accuracy of currently available serology kits in this population. The ultimate goal was to determine whether a sero-

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Table 1 UBT, histology and endoscopy findings according to patient age

	Number (%) of patients aged <50 years (n=53)	Number (%) of patients aged >50 years (n=31)	Total number (%) of patients (n=84)
UBT positive[a]	9 (26)	11 (44)	20 (33)
Histological evidence of gastritis and *H pylori* infection	15 (28)	14 (45)	29 (35)
Histological evidence of chemical gastropathy	26 (49)	11 (35)	37 (44)
Histological evidence of acute gastritis and *Gastrospirillium hominis* infection	0	1 (3)	1 (1)
No histological abnormality	11 (21)	6 (19)	17 (20)[b]
Oesophagitis seen at endoscopy	5 (9)	3 (10)	8 (10)
Ulcer seen at endoscopy	5 (9)[c]	2 (6)[d]	7 (8)

[a] Only 60 of 84 patients attended for UBT; [b] in four of these cases there was endoscopic evidence of oesophagitis; [c] includes four patients with duodenal ulcer and one patient with gastric ulcer disease; [d] includes one patient with duodenal ulcer and one patient with gastric ulcer disease.

logical diagnostic service for *H pylori* infection had the potential to reduce the increasing demands on endoscopy and nuclear medicine units.

Methods

We wrote to local general practitioners asking them to refer patients newly presenting with symptoms of moderate or severe dyspepsia (not defined) for inclusion in the study. Patients were excluded from the study if they had received any antibiotic, bismuth containing compound, or proton-pump inhibitor drug, which may have had anti-*H pylori* activity, within the previous two months. We also excluded those patients who had previously undergone upper gastrointestinal endoscopy to prevent bias towards a greater chance of being infected with *H pylori*.

ENDOSCOPY AND HISTOLOGY

All patients underwent routine upper gastrointestinal endoscopy with biopsy of the gastric antrum and other abnormal tissue where appropriate. Specimens were placed in formalin and subsequently were examined by routine histological methods and, after Giemsa staining, for bacteria with the morphological appearance of *H pylori*.

UREA BREATH TEST

Patients were given appointments to attend (usually within six weeks of endoscopy and serum sampling) for a urea breath test (UBT).[9] ^{14}C-urea (equivalent to 180 kBq of radioactivity) was administered orally in 20 ml water. Twenty minutes later patients were asked to expire through a tube containing 1 ml of 1 M hyamine in methanol until the indicator became colourless; this was equivalent to the collection of 1 mmole CO_2. Scintillant fluid was added to the CO_2 sample which was then passed through a β-particle scintillation counter. Earlier studies had indicated that recovery of >8% of the original radioactive dose was associated with *H pylori* infection (UBT positive).

SEROLOGY

During the visit to the endoscopy unit all patients had approximately 10 ml of blood taken, which was then sent to the microbiology laboratory for serum separation and storage at −20°C. *H pylori* specific IgG assays were performed using the following enzyme linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions: G.A.P. IgG (Biorad, Herts, UK); Pylori-Elisa II (Bio-Whitaker, Surrey, UK); Hp-G Screen (Genesis Diagnostics, Cambs, UK); Microstar EIA (Kenstar); Premier *H pylori* (Meridian Diagnostics Inc. supplied by Launch Diagnostics, Kent, UK); Pyloriset EIA-G (Orion Diagnostica; Murex, Kent, UK); Helico-G (Porton Cambridge, Cambs, UK); and SIA *Helicobacter pylori* (Sigma, Dorset, UK). Two of these kits are qualitative but not quantitative assays (Launch and Sigma). Serum samples were tested and the results analysed without knowledge of the results of histology or UBT. In the cases where equivocal results were obtained, as defined by respective manufacturers, repeat testing was not carried out.

DEFINITION OF *H PYLORI* INFECTED PATIENTS

Patients were considered to be infected with *H pylori* if either bacteria with typical morphology were seen by histology or the UBT was positive. The results of one gold standard technique were determined without knowledge of the other.

Results

PATIENTS AND DIAGNOSES

Eighty four patients were studied and their median (mean, range) age was 45 years (45·9, 22–79). The histological and UBT diagnoses, according to patient age, are shown in table 1. Sixty three per cent of the study group were aged <50 years; this cut off was used to analyse data because gastric malignancy is very rare in the local population below this age, and therefore endoscopy is not mandatory in such individuals. The overall prevalence of *H pylori* infection was 35%. There was no significant difference in the prevalence of any of the findings listed in table 1 according to age group, although, as expected, there was an increased likelihood of *H pylori* infection in the patients aged >50 years ($\chi^2 = 2{\cdot}46$, $p > 0{\cdot}1$).

Twenty four patients failed to attend for UBT. In the remaining 60 patients, with one exception, there was complete concordance between the results of UBT and histology in terms of the presence of *H pylori* infection. The single discordant pair of results (*H pylori* positive by histology, but *H pylori* negative by UBT) occurred in a patient for whom there was a

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Table 2 Serology results and percentage accuracy of kits

Kit	*Number seropositive (29 true positives)*	*Number seronegative (55 true negatives)*	*Number giving equivocal result (%)*	*Percentage accuracy*
Biorad	28	46	10 (12)	88
BioWhitaker	29	53	1 (1)	98
Genesis	27	50	2 (2)	92
Kenstar	28	42	6 (7)	83
Launch	29	55	0	100
Orion	28	51	0	94
Porton	28	52	0	95
Sigma	26	54	3 (4)	95

Table 3 Sensitivity, specificity and positive and negative predictive values of serology kits

Kit	*Sensitivity (true positive rate) %*	*Specificity (true negative rate) %*	*Positive predictive value %*	*Negative predictive value %*
Biorad	97	84	100	100
BioWhitaker	100	96	97	100
Genesis	93	91	84	100
Kenstar	97	76	80	98
Launch	100	100	100	100
Orion	97	93	88	98
Porton	97	95	90	98
Sigma	90	98	96	100

three month gap in between endoscopy and UBT, because of an initial failure to attend for the latter investigation. During this time, the patient completed a *H pylori* eradication regimen prescribed by his general practitioner. This should not, therefore, be regarded as a false negative UBT result.

The performances of the serological kits compared with the results of histology and UBT are shown in tables 2 and 3. In 60 (71%) patients the *H pylori* serology result was the same as the gold standard methods for all of the kits tested. This concordance rate was higher for the patients with (24/29, 83%) than for those without (36/55, 65%) *H pylori* infection ($\chi^2 = 2{\cdot}79$, $0{\cdot}1 > p > 0{\cdot}05$). Significantly, more patients aged >50 years, when compared with younger individuals, had discordant serology results (42% *v* 21%; $\chi^2 = 4{\cdot}29$, $p < 0{\cdot}05$).

While all the kits had good sensitivity (90–100%), specificity was more variable (76–100%) (table 3). There was a wide variation in the rate of equivocal results between the kits (0–12%); three of the kits, Launch, Orion and Porton, are calibrated always to yield unequivocal results. The positive and negative predictive values were generally high, but these do not take into account the serum samples which gave equivocal results. However, the percentage accuracy values (that is, the proportion of results where the serology result is not equivocal and agrees with gold standards) (83–100%) permit a clear ranking of the overall performances of the kits.

Discussion

The prevalence of *H pylori* infection in our study population was 35%. This is somewhat lower than might have been expected as seroepidemiological surveys in the UK have indicated a similar prevalence of *H pylori* infection in non-symptomatic individuals.[10] Also, three recent UK studies examined the potential of serology to reduce the workload of endoscopy units, investigating symptomatic patients for the presence of *H pylori* infection, and each found a prevalence of infection of approximately 50%.[11–13] It is not clear whether these studies included high proportions of inner city patients, and therefore individuals living in high density dwellings, where the prevalence of *H pylori* infection would be expected to be relatively high.[14] In contrast, Addenbrooke's Hospital serves an affluent rural and suburban population. The excellent concordance which we found between the results of histology and UBT indicate that our observed prevalence of *H pylori* infection is not falsely low. Indeed, our findings endorse the need for laboratories to be aware of the expected prevalences of *H pylori* in their client populations so that they can accurately assess the positive and negative predictive values of their serology test results. Lower prevalences of *H pylori* infection will be associated with lower positive and higher negative predictive values. Laboratories which provide reference services cannot easily estimate the predictive values because of their heterogeneous client populations.

Two kits performed exceptionally well, namely Pylori-Elisa II (BioWhitaker) and Premier (Launch). The performances of these kits in the present study compared favourably with those observed elsewhere. We are confident of their accuracy given the blinded nature of our study and the excellent concordance of the gold standard methods for detecting *H pylori* infection. The use of insensitive gold standard methods will inevitably result in spurious low specificity values for comparator methods. Each of the two kits was easy to use and has only minor drawbacks. The BioWhitaker kit requires the use in a spectrophotometer of a filter with a wavelength (550 nm) which is not commonly used with other ELISA kits, and hence laboratories may need to purchase one of these separately. The Launch kit was one of two enzyme immunoassay kits tested which are marketed as qualitative as opposed to quantitative assays (although a numerical result is still obtained); linear regression analysis of the values obtained by the Launch and Sigma kits compared with those obtained by the BioWhitaker kit gave r^2 values of 0·835 and 0·894, respectively ($p < 0{\cdot}0001$ in both cases). Since this study was started, the Launch kit has now been modified, involving minor changes to the way results are interpreted, to give a quantitative result.

The age specific incidence of gastric malignancy is very low in East Anglia below 50 years, rising after this age (East Anglian Cancer Registry, personal communication). We therefore used an age cut off of 50 years with which to analyse data, because below this age endoscopy is not mandatory to exclude malignancy in patients newly presenting with dyspepsia. We believe it is safe not to endoscope patients aged <50 years who present with dyspepsia, but instead to use serology to identify those with *H pylori* infection. Whether *H pylori* antibody positive, dyspeptic patients are then given eradication therapy or first examined by gastroscopy, those with persistent symptoms will be endoscoped, and so rare cases of malig-

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nancy identified in young individuals. At present, because of the marked interpatient variation, and in some cases delay of several months in the decrease *H pylori* antibodies, we cannot see a role for repeat IgG measurements in patients who have received eradication treatment.[15] The UBT is better suited to the investigation of patients who may have relapsed or been reinfected.[4]

Serology was generally more accurate in individuals aged <50 years, and in those who were infected with *H pylori* as determined by the gold standard methods, although this was only evident for some (less accurate) kits. We used the manufacturers' recommended cut off values to interpret ELISA optical density values, but it is possible that optimal cut off values for primary target populations could be determined, by generating receiver operator characteristic curves for the kits which were less accurate.[16] However, such exercises are time consuming and ideally require that at least two gold standard methods are used to validate the serology results; hence, the majority of laboratories would not be able to manipulate cut off values and would favour kits which require no pre-validation. Cross-reacting antibodies are the likely cause of false positive serology results. The rate of decrease in serum *H pylori* antibody levels after eradication treatment is variable, and it is therefore possible that some of the false positive serology results may be due to the presence of long lived antibodies in patients previously cleared of their infection. The higher discrepancy rate in the serology results of individuals aged >50 years concurs with both of these possibilities. Serum from one patient in particular gave positive serology results in five of the kits tested (plus one equivocal result); this patient was aged 44 years with no known history of peptic ulceration. Two other patient serum samples each reacted in three kits (plus an equivocal result in another kit).

The rate of equivocal results was unacceptably high with some kits (up to 12%). Although some of the manufacturers recommend retesting serum samples giving equivocal results, this creates extra work, increases assay costs, and delays the time until a satisfactory result is obtained, which may be a week for many laboratories which batch such serology assays. It is also difficult to be confident about the accuracy of a second result, assuming a repeat equivocal result is not obtained. Issuing equivocal serology results is understandably likely to frustrate the requesting doctor. As the most accurate kit which we examined (Launch) did not have a grey/equivocal zone, it is clearly possible to produce *H pylori* serology results of high positive and negative predictive value without equivocal results.

The cost of *H pylori* serology is considerably less than that of either endoscopic diagnosis or UBT (approximately £5–10 versus £150 or £40, respectively). Some, but not all, of the serology kits we examined can be considered sufficiently accurate for the primary diagnosis of *H pylori* infection in symptomatic patients, particularly in those aged <50 years. For patients aged >50 years with new symptoms of dyspepsia, serology may be used to determine whether *H pylori* infection is present and eradication treatment given, with endoscopy used to exclude malignancy. Serology is also of use in selecting patients with long-standing dyspepsia for *H pylori* eradication. We are, therefore, introducing a diagnostic *H pylori* serology service given the high degree of accuracy observed in this study.

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