Evaluation of a commercial ELISA for serodiagnosis of *Helicobacter pylori* infection

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Abstract

A commercial ELISA for the detection of *Helicobacter pylori* IgG antibodies was evaluated using serum from 242 patients attending an endoscopy clinic. The efficacy of the ELISA was assessed in relation to the histological detection of *H pylori* on antral mucosal biopsy specimens. In patients under 61 years of age (n = 138) the ELISA was 97.5% sensitive and 85.5% specific for *H pylori* infection, with a positive predictive value of 91% and a negative predictive value of 96%. Over the whole group the sensitivity of the ELISA was 93.8% and the specificity 79.3%. The positive predictive value and negative predictive values were, respectively, 90% and 87%. These results suggest that the Bio-Rad GAP IgG *H pylori* ELISA is suitable for serodiagnosis of *H pylori* infections for most clinical purposes and thus makes *H pylori* serology available to routine diagnostic laboratories.

Colonisation of the human gastric mucosa by *Helicobacter pylori* stimulates a specific systemic humoral response to the bacterium.¹⁻⁷ Although early investigations showed the presence of a systemic immune response by agglutination and complement fixation techniques,^{6,7} more recent studies have shown the suitability of the enzyme linked immunosorbent assay (ELISA) for detection of *H pylori* specific antibodies.^{1-5,8-11} Several different antigen preparations and methods have been used in these ELISAs and there has been no universal attempt to standardise assays. *H pylori* serology has thus remained largely a research tool and not generally applicable to routine diagnostic laboratories. The increasing evidence implicating *H pylori* in the aetiology of gastritis and perhaps also peptic ulcer disease¹² makes accurate diagnosis of infection by non-invasive techniques such as ELISA clinically relevant.

In this study we evaluated the first commercially available *H pylori* IgG ELISA. The efficacy of the assay was assessed in relation to the histological detection of the bacterium on the antral mucosa in a well defined group of patients.

Methods

Two hundred and forty two patients (104 men, 138 women; mean age 56.7, range 19-95)

undergoing routine upper gastrointestinal endoscopy were studied. Blood was taken immediately before endoscopy and sera were stored at -20°C until assayed. At endoscopy, biopsy specimens were taken from the gastric antrum and body using large biopsy forceps (Olympus/Keymed ref FB 13K) and immediately fixed in 4% neutral buffered formalin for histological assessment.

Specimens were routinely processed and stained with haematoxylin and eosin. The presence and severity of gastritis was assessed by one pathologist (JIW) using Whitehead's classification.¹³ Chemical gastritis was assessed according to the criteria of Wyatt and Dixon.¹⁴ *H pylori* were identified histologically with a modified Giemsa stain,¹⁵ and patients were considered positive if *H pylori* was found in any biopsy specimen. It has been shown that there is the potential for sampling error when assessing *H pylori* histologically.¹⁶ To avoid including false negative patients in the *H pylori* negative group, those in whom chronic superficial or atrophic gastritis was present, but in whom *H pylori* was not identified in any biopsy specimen, were excluded from the study. The patients selected should therefore represent "true positive" and "true negative" cases.

Serum IgG *H pylori* antibodies were measured using a semiquantitative commercial IgG ELISA for *H pylori* (Bio-Rad, GAP Test IgG) according to the manufacturer's instructions (the approximate cost per sample assayed in duplicate being £4.06). This ELISA utilises a DEAE-ion exchange chromatography purified *H pylori* antigen preparation. Serial twofold dilutions (1 in 2 to 1 in 16) of positive (P) control sera and negative (N) control sera were assayed in duplicate on each ELISA plate to determine the cut off point for semiquantitative analysis, based on a P:N ratio. Semiquantitative titres 3+, 2+, 1+, and +/- were respectively assigned to the P/N values derived from 1 in 2, 1 in 4, 1 in 8, and 1 in 16 positive control dilutions. In determining the sensitivity and specificity of the assay patients with semiquantitative titres 3+, 2+, and 1+ were considered seropositive. Patients with semiquantitative titres +/- or P/N values below that of the 1 in 16 dilution of the positive control sera were considered seronegative. Intra-assay variability for IgG was 11% and interassay variability 12.3%.

Sera of patients who were serologically positive in *H pylori* IgG ELISA but histologically negative for the bacterium were

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Table 1 Histology of antral mucosa in *H pylori* positive and negative subjects

	<i>H pylori</i> negative (n = 82)	<i>H pylori</i> positive (n = 160)
Normal	65	0
Superficial gastritis	0	43
Chronic atrophic gastritis	0	117
Chemical gastritis	16	0
Fibrosis	1	0

Table 2 Semiquantitative titre in *H pylori* IgG ELISA for *H pylori* positive and negative subjects

Semiquantitative titre	<i>H pylori</i> positive (n = 160)	<i>H pylori</i> negative (n = 82)
3+	100	5
2+	35	5
1+	15	7
+/-	4	16
Negative	6	49

further analysed for *H pylori* IgG antibodies by immunoblotting.¹⁷ The antigen preparation was a whole cell preparation of *H pylori*, strain NCTC 11637.

Results

One hundred and sixty of the 242 patients were colonised with *H pylori* and 82 patients were negative for *H pylori*. The histological diagnosis in the *H pylori* positive and negative patients is shown in table 1. None of the *H pylori* positive subjects had histologically normal antral mucosa, 43 had superficial gastritis, and 117 had chronic atrophic gastritis. In eight patients *H pylori* was identified only in biopsy specimens from the gastric body but not the antrum. All of these patients had superficial or chronic atrophic antral gastritis. In *H pylori* negative patients the antral mucosa was histologically normal in 65; 16 had chemical gastritis and one fibrosis.

Optical densities and semiquantitative titres in the *H pylori* IgG ELISA for *H pylori* positive and negative subjects are shown, respectively, in the figure and table 2. Ten of the 160 histologically positive subjects were serologically negative (+/- or negative). Conversely, 17 of the 82 histologically negative subjects were serologically positive with semiquantitative titres of 3+, 2+, or 1+. Three of the 17 seropositive patients had a histological picture of chemical gastritis; these three patients were also positive by immunoblotting for *H pylori* IgG antibodies, while all 14 seropositive patients with normal histology were negative by immunoblotting (data not shown). The sensitivity of the ELISA is 93.8% and the specificity 79.3%. The assay has a positive predictive value of 90% and a negative

predictive value of 87% (table 3). Exclusion of the three histologically negative patients who were positive by immunoblotting from analysis improves the assay specificity to 82.3%.

Our recent studies have shown that in the elderly the correlation between seropositivity and histological evidence of colonisation with *H pylori* is poor. Because serology is a possible tool for screening younger dyspeptic patients, the results were also analysed to include only patients under 61 years of age (n = 138). The semiquantitative titres for these patients in the *H pylori* ELISA are shown in table 4. Only two of the *H pylori* positive patients were serologically negative and eight of the *H pylori* negative subjects were serologically positive. For patients under 61 years of age, the sensitivity of the ELISA was 97.5% and the specificity 85.5%, the positive predictive value 91% and the negative predictive value 96% (table 3).

Discussion

In this study we assessed a commercial *H pylori* IgG ELISA with respect to histological evidence of *H pylori* colonisation of the antral mucosa. Histological detection of *H pylori* in gastric biopsy specimens compares well with other techniques,¹⁸ and in particular does not seem to give false positive results. It has the added advantage of permitting concurrent assessment of gastritis in biopsy specimens. In evaluating serological positivity against histological positivity, however, one must take account of the possibility of false negative histology resulting from patchy infection. Alternative validation procedures, such as bacterial culture or urease testing, would be subject to the same variability.¹⁸ Conversely, histologically positive patients with no systemic *H pylori* IgG antibodies could be subjects in the early stages of infection.¹⁹

The sensitivity and specificity of any *H pylori* IgG ELISA are strongly influenced by the chosen "cut off" point as well as the antigen preparation used. A high "cut-off" titre improves the specificity but reduces the sensitivity; conversely, a low titre "cut-off" increases the sensitivity but reduces the specificity.³

The sensitivity and specificity obtained with the commercial *H pylori* IgG ELISA in this study compare favourably with the results of other non-commercial *H pylori* ELISAs. The reported sensitivities and specificities for *H pylori* IgG ELISAs vary according to the antigen preparation used. Sensitivity with an acid glycine extract of *H pylori* ranged from 81–

Optical densities of *H pylori* negative and positive patients in *H pylori* IgG ELISA.

Table 3 Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values for Bio-Rad IgG *H pylori*

	Sensitivity	Specificity	PPV	NPV
Age range 19–95 years (n = 242)	93.8%	79.3%	90%	87%
Under 61 years (n = 138)	97.5%	85.5%	91%	96%

Table 4 Semiquantitative titre in *H pylori* IgG ELISA for *H pylori* positive and negative subjects under 61 years of age

Semiquantitative titre	<i>H pylori</i> positive (n = 83)	<i>H pylori</i> negative (n = 55)
3+	58	1
2+	17	2
1+	6	5
+/-	0	12
Negative	2	35

99% and specificity from 78–97%.³ ELISAs using a crude urease preparation showed improved sensitivity (100%) but reduced specificity (79%).²⁰ Sonicated whole cell *H pylori* antigens have a specificity of 90% and a sensitivity of 96.5% when assessed against a small sample of culture positive patients with histological gastritis, assessment against histological gastritis alone giving a specificity of 92.9% and a sensitivity of 73.2%.¹⁰ Recently *H pylori* ELISAs based on high molecular weight cell-associated proteins have been described which are 98.7% sensitive and 100% specific.¹¹ Inclusion of a 120 kilodalton *H pylori* protein with ultracentrifuged sonicate antigen preparations or acid glycine extracts improved both sensitivity (97%) and specificity (100%).²¹

The only histologically negative patients who were seropositive by ELISA and immunoblotting but in whom no *H pylori* were seen had the histological changes characteristic of “chemical gastritis” in their antral biopsy specimens. The strongly positive results by immunoblotting confirm that these three patients had a true systemic response against the bacterium. The discrepancy might be explained either by a patchy infection, or, alternatively, the histology of “chemical gastritis” may perhaps be mimicked by the healing stage of *H pylori* gastritis. Exclusion of these patients from data analysis would improve the assay specificity.

Although seropositivity for *H pylori* increases with age,⁷ the association between seropositivity and colonisation in the elderly seems to be less clear. Increasing hypochlorhydria with age can be associated with bacterial overgrowth.²² Reduced immunological responses in the elderly are well documented.²³ Consideration of age profiles is therefore important when assessing *H pylori* ELISA sensitivity and specificity. Our own data show improved sensitivity and specificity of the commercial *H pylori* ELISA for patients under 61 years old. Some studies evaluating the sensitivity and specificity of *H pylori* IgG ELISAs, however, give no age details of patients included.^{8,10} As one major role for *H pylori* serology is likely to be screening younger dyspeptic patients to eliminate unnecessary endoscopies,²⁴ evalua-

tion of the assay efficacy in younger patients is more clinically relevant.

H pylori serology represents a rapid, non-invasive test for determining colonisation of *H pylori*. The availability of a commercial ELISA with high sensitivity and specificity for *H pylori* makes it applicable now for use in routine diagnostic laboratories.

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