

RELIABILITY OF TWO COMMERCIAL SEROLOGICAL KITS FOR SERODIAGNOSING *HELICOBACTER PYLORI* INFECTION

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Abstract. Two commercial serological kits, Pylori-set (Orion Diagnostica, Finland) and Helico-G (Cambridge Biomedical Ltd, UK), and an in-house ELISA were evaluated with sera from 24 *Helicobacter pylori*-positive and 146 *H. pylori*-negative dyspeptic patients. Sensitivity, specificity, positive and negative predictive values of Pylori-set were lower than that of Helico-G and in-house ELISA. Helico-G was more sensitive (91.7%) than in-house ELISA (83.3%) and both had comparable negative predictive values of 98.3% and 97.3%, respectively. However, specificity (97.9%) and positive predictive value (86.9%) of an in-house ELISA were much higher than specificity (80.1%) and positive predictive value (43.1%) of Helico-G. Kappa index of agreement between the three serological tests (Pylori-set, Helico-G or in-house ELISA) and the presence of *H. pylori* in antral biopsies was very low ($k=0.13$; $z=1.9$; $p>0.05$), moderate ($k=0.49$; $z=7.1$; $p<0.0001$), or substantial ($k=0.82$; $z=10.8$; $p<0.0001$), respectively. Overall, statistical evaluations demonstrated that both commercial kits were not as reliable as the in-house ELISA for serodiagnosing *H. pylori* infection.

INTRODUCTION

Invasive procedures such as direct urease testing, histological examination and culturing of the endoscopic antral biopsy specimens (Schnell and Schubert, 1989) or non-invasive techniques such as urea breath tests (Graham *et al.*, 1987; Marshall *et al.*, 1991), and serology (Newell *et al.*, 1988) have been used in the diagnosis of *Helicobacter pylori* infection. However, the breath tests use radioactive or isotopic urea and require relatively expensive equipments such as scintillation counter or mass spectrophotometer, respectively.

In contrast to the invasive and technically demanding diagnostic procedures, specific detection of serum antibodies directed against the bacterium offers a non-invasive and relatively simple procedure for serodiagnosis of suspected infection. The present study was undertaken with the objectives: firstly, to assess the efficacy of two commercial serological kits, Pylori-set (Orion Diagnostica, Finland) and Helico-G (Cambridge Biomedical Ltd, UK), and in-house ELISA in relation to the presence of *H. pylori* in antral biopsies of a selected group of patients; and secondly, to evaluate their use for serodiagnosing *H. pylori* infection.

MATERIALS AND METHODS

One hundred and seventy consecutive dyspeptic patients (Males 91; Females 79) referred for gastroduodenoscopy were studied during a 20-month period from January 1991 to August 1992. The ages of the patients ranged from 15 to 83 years (median 45 years). Patients were instructed to fast overnight prior to endoscopy.

At endoscopy, between 3 to 5 biopsies were taken from the antrum of each patients for microbiological detection of *H. pylori* and histopathology. For microbiological analysis, the urease test and impression smear were performed immediately at the endoscopy suite. The former was performed by inoculating a fresh biopsy specimen in a tube of Christensen's urea broth (Oxoid, Basingstoke, UK) supplemented with 2% (w/v) urea (Sigma Chem, St Louis, USA). For impression smear, a fresh biopsy tissue was gently rolled over a clean glass slide with a hypodermic needle. After air dried, the smear was stained according to Gram's procedure using carbol fuchsin as the counter stain. Biopsy tissue for culture was transported in Stuart transport medium (Oxoid, Basingstoke, UK) and processed within 2 hours of collection. After ma-

ceration in 200 µl phosphate buffered saline (PBS) pH 7.2 (Behringwerke AG, Marburg, W. Germany), 50 µl aliquots were each streaked onto: (1) two plates of Eugon agar medium supplemented with 10% (v/v) human blood and Dent antibiotic supplement (Oxoid, Basingstoke, UK), and: (2) 2 plates of the same medium but without the Dent antibiotic supplement. Two plates (with and without antibiotic supplement) were then placed in an anaerobic jar containing a commercial microaerophilic gas generating kit (Oxoid, Basingstoke, UK), whereas another pair were placed in a 10% carbon dioxide-air atmosphere. All plates were incubated at 37°C, and examined after 5 to 7 days. *H. pylori* was identified on the basis of cell morphology, negative Gram staining reaction, production of urease, catalase and oxidase. The "gold standard" for *H. pylori* infection was defined as either positive by culture or positive by both urease test and Gram stain (Kelkar *et al.*, 1990). For histopathology, the fresh biopsy specimens were transported in formol-saline, embedded in paraffin, sectioned and stained by Haematoxylin and Eosin and Warthin Starry methods. The presence of chronic gastritis or active chronic gastritis (Morris *et al.*, 1988) was a qualitative assessment subject to the judgment of the individual pathologist.

Immediately before endoscopy, all patients had blood withdrawn for serological testing. After centrifugation, serum was stored in aliquots at -70°C. Pylori-set was performed by mixing serum (diluted 1 : 2) from each patient with the test latex reagent containing the *H. pylori* antigen. Within 3 minutes, the result was considered negative when agglutination was not detected or positive when agglutination was detected. These results were read by comparing with the negative or positive controls included in each test kit.

For Helico-G, 100 µl of patient serum diluted 1 : 200 in diluent containing 0.1% (w/v) sodium merthiolate (other specifications not given) was added to the wells. After incubation for 1 hour at 37°C, each well was washed three times with the diluent. After the wells were dried, 100 µl of goat anti-human IgG peroxidase conjugate was added and incubated for 30 minutes at 37°C. After the wells were washed three times and dried, 100 µl of substrate (stabilised mixture of tetramethyl benzidine and urea peroxide) was added to each well and incubated at room temperature for 10 minutes. Fifty microliters of 2M sul-

phuric acid were then added to each well to stop the color reaction. Optical density was read at 450 nm using microplate reader (Anthos labtec instruments). Two wells each of diluent blank, calibrator containing 10 U/ml of anti-*H. pylori* IgG and calibrator containing 100 U/ml of anti-*H. pylori* IgG were included in each plate during the assay. A positive result was recorded if sample optical density reading was equal to or higher than that of the 10 U/ml calibrator. Acid glycine extract (Newell *et al.*, 1988) of *H. pylori* was used as the antigen for in-house ELISA. Protein content of the extract was determined using standard method (Bio-rad, Richmond, CA) with bovine serum albumin as a standard. The optimum quantity of the antigen and serum dilution determined with an ELISA by block titration using the pooled positive and pooled negative control sera was 2.5 µ/ml and 1 : 256, respectively. In-house ELISA was performed according to Newell (1987) except that we used PBS with 0.05% (v/v) Tween-20 (PBS-T) for washing and the diluting the antibodies. Goat anti-human IgG peroxidase conjugate (ICN, Biomedical lab) was diluted 1 : 1,000 and ortho-phenylene diamine (Sigma Chem, St Louis, USA) in phosphate citrate buffer and H₂O₂ was used as color development reagent. A positive result was defined as an optical density of > 2 standard deviation above the mean value for 146 patients who were negative for *H. pylori* infection. Two-tailed Fischer exact test was computed with Epistat statistical package (Beaver Software Inc). The sensitivity, specificity, negative predictive value and positive predictive value of the tests were calculated according to Newell *et al.* (1988). Kappa (k) statistical method (Fleiss, 1973) was used for a more accurate evaluation of the index of agreement.

RESULTS

In comparison with the "gold standard", the sensitivity for the detection of *H. pylori* was 91.3% by urease test or by Gram staining of impression smear, and 87.0% by culture (Table 1). All the tests gave high positive predictive values (95.5%-100%) and high negative predictive values (98.0%-98.6%). The prevalence rate of *H. pylori* infection obtained in this study was low at only 14.1% (24/170).

Table 2 compared the results from Pylori-set, Helico-G and in-house ELISA to serodiagnose *H.*

Table 1

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of different diagnostic tests for the detection of *H. pylori* in antral biopsy specimens of 170 dyspeptic patients.

Tests	No. of biopsies	True positive	False positive	True negative	False negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Urease	160	21	1	136	2	91.3	99.3	95.5	98.6
Gram staining	168	21	0	145	2	91.3	100.0	100.0	98.6
Culture	168	20	0	145	3	87.0	100.0	100.0	98.0

Table 2

Comparison of results from Helico-G, Pylori-set and in-house ELISA with the presence *H. pylori* in antral biopsies of 170 dyspeptic patients.

Possible results	Helico-G/Pylori-set	Helico-G/In-house ELISA	Pylori-set/In-house ELISA
-/-	93 (2)	118 (2)	111 (4)
-/+	26 (0)	1 (0)	9 (9)
+/-	27 (11)	29 (2)	36 (0)
+/+	24 (11)	22 (20)	14 (11)

Number in parenthesis indicates true positive cases which were confirmed microbiologically.

pylori infection in 170 dyspeptic patients. Pylori-set, Helico-G and in-house ELISA detected *H. pylori* in 11, 22 and 20 of the 24 microbiologically confirmed positive cases, respectively. On further analysis, 11 of the 50 Pylori-set positive patients, 22 of the 51 Helico-G positive patients, and 20 of the 23 in-house ELISA positive patients were *H. pylori* positive.

Adequate histological diagnosis were available in 162 patients. The remaining 8 patients without adequate histological diagnosis consists of 2 *H. pylori*-positive and 6 *H. pylori*-negative patients. *H. pylori* was present in 2.2% (2/92), 16.3% (7/43) and 48.1% (13/27) of patients with histologically normal mucosa, chronic gastritis or active chronic gastritis, respectively. There was a strong association between *H. pylori* infection and chronic gastritis ($p < 0.005$) or between *H. pylori* and active chronic gastritis ($p < 0.00001$).

Over the whole group, the sensitivity, specificity,

positive predictive and negative predictive values of Pylori-set for the detection of *H. pylori* were lower than that of Helico-G and in-house ELISA (Table 2). Helico-G was more sensitive (91.7%) than in-house ELISA (83.3%), and both had comparable negative predictive values of 98.3% and 97.3%, respectively. However, the specificity (97.9%) and positive predictive value (86.9%) of in-house ELISA were much higher than the specificity (80.1%) and positive predictive value (43.1%) of Helico-G. The sensitivity and specificity of Pylori-set and Helico-G were lower than those claimed by the manufacturer. In a more accurate statistical analysis, kappa index of agreement between Pylori-set and the "gold standard" was very low ($k = 0.13$; $z = 1.9$; $p > 0.05$), while it was moderate between Helico-G and the "gold standard" ($k = 0.49$; $z = 7.1$; $p < 0.00001$), and substantial between in-house ELISA and the "gold standard" ($k = 0.82$; $z = 10.8$, $p < 0.0001$) (Table 3).

Table 3

Comparison of the sensitivity, specificity, predictive values, and agreement, and kappa indexes between Helico-G, Pylori-set and in-house ELISA with the "gold standard"

Assays	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Observed agreement (%)	Kappa index (x, p-value)
Helico-G vs gold standard	91.7 (22/24)	80.1 (117/146)	43.1 (22/51)	98.3 (117/119)	81.8 (139/170)	0.49 (7.1, $p < 0.0001$)
Pylori-set vs gold standard	45.9 (11/24)	73.2 (107/146)	22.0 (11/50)	89.2 (107/120)	69.4 (118/170)	0.13 (1.9, $p > 0.05$)
In-house ELISA vs gold standard	83.3 (20/24)	97.9 (143/146)	86.9 (20/23)	97.3 (143/147)	95.9 (163/170)	0.82 (10.8, $p < 0.0001$)

DISCUSSION

Evaluation of serological tests to serodiagnose *H. pylori* infection depends on whether infection is truly present or not. The use of urease test, Gram staining of impression smear and culture for the detection of *H. pylori* have been reported. Our findings that urease test, microscopy and culture gave a sensitivity of 91.3%, 91.3% and 87.0%, respectively are comparable with others (Westblom *et al.*, 1988; Parsonnet *et al.*, 1988; Schnell and Schubert, 1989). There have been reports that distribution of *H. pylori* in the antrum is patchy (Goodwin *et al.*, 1986). Therefore, in some truly positive cases, *H. pylori* could inevitably have been missed. However, the high sensitivity, specificity and predictive values obtained in this study suggest that though we may have slightly under detected *H. pylori*, under detection may not be a major factor in accounting for the low prevalence rate obtained. The prevalence rate at 14.1% was lower compared with another study in this country (Goh *et al.*, 1990), but in agreement with other studies elsewhere (Satti *et al.*, 1990), we found strong association between *H. pylori* and chronic gastritis ($p < 0.005$) or between *H. pylori* and active chronic gastritis ($p < 0.00001$). In our study, only 2.2% of histologically normal dyspeptic patients harboured the bacterium. Therefore, the "gold standard" chosen is less likely to alter significantly the observed sensitivity, specificity and predictive values obtained for the three serological tests.

In this study, two commercial kits and in-house ELISA were assessed in relation to the presence of *H. pylori* in antral biopsies. Our finding that Pylori-set and Helico-G had lower positive predictive

value than in-house ELISA (Table 3) were indicative, probably, that both kits detected false-positive results. Such results override their advantage as rapid non-invasive serological kits. Additionally, we also found that kappa index of agreement between in-house ELISA and the "gold standard" was good ($k = 0.82$), while it was low ($k = 0.13$) or moderate ($k = 0.49$) between Pylori-set or Helico-G and the "gold standard", respectively. Acceptable serodiagnostic kits for any infection must be reliable, economical, rapid and easy to perform. A simple latex agglutination (*eg* Pylori-set) and an enzyme-linked immunosorbent assay (*eg* Helico-G) could mean new possibilities for serodiagnosis in countries where financial and endoscopic resources are limited. Screening with an IgG ELISA has been shown to reduce endoscopy work load by 23.3% while maintaining a sensitivity of 97.4% for detecting peptic ulcer disease (Sobala *et al.*, 1991). Though both the commercial kits are economical, rapid and easy to perform, accurate kappa statistical analysis revealed that they were not as reliable as the in-house ELISA for serodiagnosing *H. pylori* infection.

Only 4.7% (8/170) of our patients were <21 years of age and they were found to be *H. pylori*-negative. Westblom *et al.* (1992) concluded that Pylori-set was inadequate for diagnosing *H. pylori* infection in pediatric patients (<21 years) but could be an important alternative to other more time-consuming diagnostic tests for *H. pylori* infection in adult patients. Contrary to their findings, Pylori-set was inadequate for serodiagnosing *H. pylori* infection in our predominantly adult patients. We retested 76 serum samples using other lots of the Pylori-set kits and obtained identical

results suggesting that the performance of the kit was consistent. Pylori-set measures total antibody. One possibility for the disagreement between our results and these of Westblom *et al.* (1992) could be age related antibody class response against antigens of *H. pylori* which may differ in different populations.

We retested 56 serum samples using a different batch of the Helico-G and obtained identical results, suggesting that, like Pylori-set, the performance of Helico-G was consistent. Sensitivity and specificity of an ELISA with an acid glycine extract of *H. pylori* had been reported to range from 81% to 99% and 78% to 97%, respectively (Goodwin *et al.* 1987). In this regard, the sensitivity and specificity of our in-house ELISA at 83.3% and 97.9%, respectively, were comparable with the reported range. It is noteworthy that the performance of in-house ELISA was more reliable than the commercial ELISA or latex agglutination kits. The obvious difference between in-house ELISA and commercial kits seems to be in the strains used and the way antigens are prepared. Therefore comparison of the performance of in-house ELISA using antigens prepared from a local strain and from the strain used commercially could possibly explain the discordant results obtained by the three serological tests.

Diagnosing *H. pylori* infection by serology is economical, rapid, easy and may prevent use of invasive procedures. However, commercial serological kits should be further evaluated before they are used routinely.

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