Diagnostic efficiency of an ultrarapid endoscopy room test for *Helicobacter pylori*

A V Thillainayagam, A S Arvind, R S Cook, I G Harrison, S Tabaqchali, M J G Farthing

Abstract

There are several reliable methods for detecting *Helicobacter pylori* but all have as a major disadvantage the time required to yield a definitive result. We have modified the standard urease test so that a positive result is available in one minute. We describe the use of this test in biopsy specimens from the gastric antrum from 220 consecutive patients referred for upper gastrointestinal endoscopy. The performance of the 'one minute test' was compared with a standard urease test, Gram stain, and microbiological culture. Using culture as the 'gold standard' 80 (36%) of the patients were *H. pylori* positive. The one minute test produced no false positives and showed a sensitivity of 89% and specificity of 100%, while the predictive values of a positive and negative test were 100% and 94% respectively. The diagnostic efficiency of the test was 96% compared to 85% for both the Gram stain and direct urease test. All positive results using the one minute test were available within one minute. The test is easy to prepare and costs approximately £0.04. This new modification of the urease test should be superior to the currently available urease tests because a reliable result will be available in almost 90% of infected individuals even before endoscopy is completed.

A variety of methods have been developed for the detection of *Helicobacter pylori* in gastric mucosa.¹ Non-invasive methods currently available are serodiagnosis and a breath test using isotopically labelled urea, but these are not yet in widespread clinical use. Although of high sensitivity, specificity, and predictive value, the breath tests²⁻⁵ may be limited by expense and, when [¹⁴C]-urea is used, radiation exposure. Serological assays have been hindered by a relatively high number of false negatives and false positives,⁶ although two new enzyme linked immunosorbent assays (ELISA) seem to have improved precision.^{7,8} These non-invasive methods will probably prove useful in unravelling the natural history of *H. pylori* infection, and may come to play a part in posttreatment surveillance programmes. For many, though, they are not available in daily clinical practice.

Several approaches to diagnosis using gastric (usually antral) biopsy specimens have been described but they all have a major disadvantage in common which is the time required to get a definitive result. Histological examination of biopsy sections using a variety of stains⁹⁻¹⁶ has been shown to be quicker and simpler than the Warthin-Starry silver stain used by Warren and Marshall,¹⁷⁻²⁰ but at best the results will not be

available from several hours to two to three days after endoscopy.²¹ Histological methods seem to have a lower specificity than culture because of the possible presence of other spiral and *Campylobacter* like organisms which can be found in the human stomach.^{22,23} Two recent immunohistological innovations seem to be capable of yielding results in under two hours^{24,25} but neither as yet has been applied outside the research laboratory. The other diagnostic 'gold standard' apart from the histological demonstration of *H. pylori* in biopsy specimens is culture of the organism, but this can take three to six days for the results to be known.²¹

An unusual characteristic of *H. pylori* is rapid urea hydrolysis due to the presence of large amounts of preformed urease in the organisms. The first description of this phenomenon by Langenberg *et al.*²⁶ was soon followed by its application to the diagnosis of *H. pylori* in biopsy specimens.²⁷ Christensen's 2% urea broth was used as enzyme substrate and ammonia production detected by the colour change induced in a pH indicator. In this initial study only 50% of biopsy specimens containing *H. pylori* were positive by six hours, some requiring up to 24 hours to yield a positive result. Since then many modified versions of the biopsy urease test have been described, in an attempt to increase speed and reliability.²⁸⁻³³ But, with even the best of these, at least 20 minutes is required for a definite positive result.

We have used preformed urease in *H. pylori* to detect the organism in gastric biopsy specimens but found that changing the incubation conditions permitted ultrarapid diagnosis, the results of which are available in the endoscopy room even before the instrument is removed from the patient.³⁴ We have now extended these studies and compared the results of this rapid test with other standard techniques for detecting *H. pylori*.

Methods

Two antral biopsy specimens were taken from within 5 cm of the pylorus from 220 consecutive patients (110 men) referred for oesophagogastrroduodenoscopy (Olympus GIF XQ10). The mean age of the patients was 56 years (range 19-94 years). This study was approved by the Ethical Committee of the City & Hackney Health District.

MICROBIOLOGICAL CULTURE, GRAM STAIN, AND STANDARD UREASE TEST

One biopsy specimen was transported to the microbiology laboratory attached to the inside of a sterile bijoux bottle containing approximately

Departments of
Gastroenterology
A V Thillainayagam
A S Arvind
M J G Farthing
and Medical
Microbiology, St
Bartholomew's Hospital,
West Smithfield, London
EC1A 7BE
R S Cook
I G Harrison
S Tabaqchali

Correspondence to:
Dr M J G Farthing.

Accepted for publication
3 July 1990

0.5 ml sterile saline. All specimens were processed within two hours of receipt. Using a sterile swab, the biopsy specimen was smeared on to a sterile glass slide for Gram staining and using the same swab the biopsy specimen was then smeared and inoculated onto a selective medium (Oxoid BAB No 2 containing Skirrow's formula for selective supplement) ensuring maximum contact.³⁵ The remainder of the biopsy was then placed into 0.5 ml of Christensen's 2% urea broth at 37°C.³⁶ The culture plates were then incubated at 37°C under microaerophilic conditions using Campybak envelopes in Gas Pak jars (BBL Microbiology Systems, Becton Dickinson). The cultures were examined after four and seven days. Colonies were identified as *H. pylori* by their characteristic morphology on Gram stain, oxidase positivity, and strong production of urease.

IN VITRO PRODUCTION OF UREASE

One *H. pylori* strain isolated from an antral biopsy specimen was used to test urea production in vitro. Unbuffered solutions of urea in deionised water at a pH of 6.8 were prepared and to 0.5 ml aliquots thereof were added one to two drops of a 1% phenol red (free acid) solution. Using a range of concentrations of *H. pylori*, we were able to show an almost immediate colour change from yellow to pink when 2×10^6 or more bacteria were added to a 0.5 ml aliquot of urea solution. The colour change occurred instantaneously over a range of urea concentrations from 0.025–10% (w/v).

ONE MINUTE UREASE TEST

An antral mucosal biopsy specimen was placed immediately into a capped Eppendorf tube containing 0.5 ml freshly prepared 10% urea (w/v) in deionised water at a pH of around 6.8 to which had been added two drops of 1% phenol red (free acid) as a pH indicator. A positive result was recorded if there was a colour change from yellow to pink within the first minute. Although the ideal pH for urease activity is 8.2,³⁶ we have been unable to improve on the rapidity of the test by using other pH indicators such as m-cresol (pH range 7.4–9.0) and thymol blue (pH range 8.0–9.6).

STATISTICAL ANALYSIS

The results were compared using the 'comparison of proportions from many samples' method.³⁷

Results

Microbiological culture grew *H. pylori* from 80 (36%) of the 220 patients. These patients are representative of the workload of most endoscopy units, with *H. pylori* being present more commonly in patients over the age of 45 years (Table I) and closely associated with peptic ulcers.

The 'one minute test' correctly identified 71 of the 80 *H. pylori* positive specimens and there were no false positives (Table II). Gram stain detected 66 of the 80 positive specimens but there were 10

false positive results as well as a greater number of false negatives. The conventional urease test also performed less reliably than the one minute test. Although there were 86 positive results, 20 were false positives, due almost certainly to the presence of other urease producing bacteria.³⁸ In addition, there were 13 false negatives. The one minute test had a comparable sensitivity to the conventional urease test (89% v 83%) but had a greater specificity (100% v 86%; $p < 0.001$). This makes the predictive value of a positive one minute test 100% and that of a negative test 94% – that is, greater than both the Gram stain and the conventional urease test ($p < 0.05$). The diagnostic efficiency of the one minute test was also significantly greater than the others ($p < 0.001$). The major clinical advantage of the one minute test was that the result was available immediately, whereas Gram stain took 1–3 hours, conventional direct urease test up to 24 hours, and culture four to seven days.

Discussion

As many patients with dyspepsia are investigated by endoscopy it seemed appropriate to develop a rapid diagnostic test for *H. pylori*, the results of which would be available in the endoscopy room immediately after the examination. We have made relatively minor changes to the well established urease test for *H. pylori* but now have a diagnostic approach that yields a positive result in almost 90% of infected patients within one minute of retrieving the specimen. All other invasive and non-invasive methods for detecting *H. pylori* take considerably longer and thus may not be available to the clinician during the endoscopy consultation (Table III). We think the main reason our modification produces more rapid results is that the urea in water solution, unlike Christensen's urea broth, is unbuffered. Thus any changes in pH will be detected quickly through their effect on the pH indicator. Our modification also has the advantage that, unlike

TABLE I Details of patients' age and *H. pylori* status

Age (years)	<i>H. pylori</i> status	
	Positive: No (%)	Negative: No (%)
Men		
<45	8 (26)	23 (74)
>45	35 (44)	44 (56)
Women		
<45	10 (33)	20 (67)
>45	27 (34)	53 (66)
Total	80 (36)	140 (64)

TABLE II Comparative efficiency of diagnostic tests for *H. pylori*

Test	Culture	One minute	Gram stain	Direct urease
Positive	80	71	66	86
Negative	140	149	154	134
False positive	–	0	10	20
False negative	–	9	23	13
Sensitivity (%)	–	89*	70	83
Specificity (%)	–	100§	94	86
Positive (%)	–	100†§	85	77
predictive value				
Negative (%)	–	94‡	85	90
predictive value				
Efficiency (%)	–	96†§	85	85

One minute v Gram stain * $p < 0.01$; † $p < 0.001$; ‡ $p < 0.05$; one minute v direct urease § $p < 0.001$.

TABLE III Comparative speeds of other available diagnostic tests for *H. pylori*

Method (and reference)	Time to positive result
CLO test ^{28,29}	20 min–24 h
Microtitre biopsy urease test ³¹	1–24 h
4 Hour rapid urease test ³²	2–4 h
6% Rapid urease test ³³	20 min–2 h
Modified biopsy urease test ³³	1–24 h
Enzyme linked immunosorbent assay ^{46–48}	4–6 h
Immunostaining ²⁵	Approx 2 h
¹³ C-urea breath test ^{1,5}	>30 min
¹³ C-urea breath test ²	>60 min

the conventional urease test, there were no false positives. Other bacteria also produce urease, albeit in much smaller quantities,^{36,38} but under prolonged incubation in the conventional urea broth this seems to be sufficient to effect a colour change. The one minute test will miss approximately 10% of *H. pylori* positive patients and one can therefore make a strong argument for back up microbiological culture or histology. Gastritis and *H. pylori* colonisation can be patchy, although usually in body type mucosa rather than in the antrum.³⁹ Taking two antral biopsy specimens may therefore reduce sampling error considerably and improve even further the sensitivity of the one minute test.

The test solutions are easy to prepare even for a gastroenterologist, and a single test costs less than £0.04. Large batches can be prepared at one time but should be stored in a freezer and only thawed before use (unpublished observations).

Thus the one minute test has practical advantages for clinical gastroenterologists who require a rapid, accurate, sensitive, and specific method of diagnosing *H. pylori* infection in patients with dyspepsia. In more than 90% of infected patients the presence of *H. pylori* can be detected in the endoscopy room even before the instrument is withdrawn. This may help in planning therapeutic strategies, lead where appropriate to prompt introduction of treatment, and possibly reduce recall outpatient appointments.

MJGF is a Wellcome Trust Senior Lecturer and gratefully acknowledges the financial support of the Wellcome Trust. We are indebted to Ms Nicola Herrera for her secretarial assistance.

- Megraud F. Comparison of different tests for *Campylobacter pylori*. *Scand J Gastroenterol* 1988; 23 (Suppl 142): 64–8.
- Graham DY, Klein PD, Evans DJ Jr, et al. *Campylobacter pylori* detected noninvasively by the ¹³C-urea breath test. *Lancet* 1987; i: 1174–7.
- Bell GD, Weil J, Harrison G, et al. ¹³C-urea breath analysis, a non-invasive test for *Campylobacter pylori* in the stomach. *Lancet* 1987; i: 1367–8.
- Marshall BJ, Surveyor I. Carbon-14 urea breath test for the diagnosis of *Campylobacter pylori* associated gastritis. *J Nucl Med* 1988; 29: 11–6.
- Rauws EAJ, Royen EAV, Langenberg W, Woensel JV, Vrij AA, Tytgat GN. ¹³C-urea breath test in *C. pylori* gastritis. *Gut* 1989; 30: 798–803.
- Goodwin CS, Blincow E, Peterson G, et al. Enzyme-linked immunosorbent assay for *Campylobacter pyloridis*: correlation with presence of *C. pyloridis* in the gastric mucosa. *J Infect Dis* 1987; 155: 488–94.
- Evans DJ Jr, Evans DG, Graham DY, Klein PD. A sensitive and specific serological test for detection of *Campylobacter pylori* infection. *Gastroenterology* 1989; 96: 1004–8.
- Dent JC, McNulty CAM, Uff JS, Gear MWL, Wilkinson SP. *Campylobacter urease*: a new serological test. *Lancet* 1988; i: 1002.
- Zarate JO, Lucero RS, Espiniella F, Padorno L, Apud A. Localization of piloricus *Campylobacter* and its relation with quantity and type of gastritis. Histological staining variants. *Dig Dis Sci* 1986; 31: 150S.
- Trowell JE, Yoong AKH, Saul KJ, Gant PW, Bell GD. Simple half-Gram stain for showing the presence of *Campylobacter pyloridis* in sections. *J Clin Pathol* 1987; 40: 702.
- Westblom TU, Madan E, Kemp J, Subik MA, Tseng J. Improved visualisation of mucus penetration by *Campylobacter pylori* using a Brown-Hopps stain. *J Clin Pathol* 1988; 41: 232.
- Gray SF, Wyatt JJ, Rathbone BJ. Simplified techniques for identifying *Campylobacter pyloridis*. *J Clin Pathol* 1986; 39: 1280.
- McMullen L, Walker MM, Bain LA, Karim QN, Baron JH. Histological identification of *Campylobacter* using Gimenez technique in gastric antral mucosa. *J Clin Pathol* 1987; 4: 464–5.
- Walters LL, Budin RE, Paull G. Acridine orange to identify *Campylobacter pyloridis* in formalin fixed paraffin-embedded gastric biopsies. *Lancet* 1986; i: 42.
- Burnett RA, Brown IL, Findlay J. Cresyl fast violet staining method for *campylobacter* like organisms. *J Clin Pathol* 1987; 40: 353.
- Peterson WL, Lee E, Feldman M. Relationship between *Campylobacter pylori* and gastritis in healthy humans after administration of placebo or indomethacin. *Gastroenterology* 1988; 95: 1185–97.
- Warren JR. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; i: 1273.
- Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; i: 1273–5.
- Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; i: 1311–5.
- Marshall BJ, Royce H, Annear DI, et al. Original isolation of *Campylobacter pyloridis* from human gastric mucosa. *Microbios Lett* 1984; 25: 83–8.
- Marshall BJ, McGeechie BD, Rogers PA, Glancey RJ. Pyloric *Campylobacter* infection and gastroduodenal disease. *Med J Aust* 1985; 142: 439–44.
- Kasper G, Dickgiesser N. Isolation from gastric epithelium of *Campylobacter*-like bacteria that are distinct from *Campylobacter pyloridis*. *Lancet* 1985; i: 111–2.
- Dent JC, McNulty CAM, Uff JC, Wilkinson SP, Gear MWL. Spiral organisms in the gastric antrum (abstr). *Lancet* 1987; ii: 96.
- Engstrand L, Pahlson C, Schwan A, Gustavsson S. Monoclonal antibodies for detection of *Campylobacter pylori* in biopsy smears and frozen section. *Scand J Gastroenterol* 1988; 23 (Suppl 142): 50–2.
- Negrini R, Lisato L, Cavazzini L, et al. Monoclonal antibodies for specific immunoperoxidase detection of *Campylobacter pylori*. *Gastroenterology* 1989; 96: 414–20.
- Langenberg ML, Tytgat GN, Schipper MEI, Rietra PJGM, Zanen HC. *Campylobacter*-like organisms in the stomach of patients and healthy individuals. *Lancet* 1984; i: 1348.
- McNulty CAM, Wise R. Rapid diagnosis of *Campylobacter*-associated gastritis. *Lancet* 1985; i: 1443–4.
- Morris A, McIntyre D, Rose T, Nicholson G. Rapid diagnosis of *Campylobacter pyloridis* infection. *Lancet* 1986; i: 149.
- Marshall BJ, Warren JR, Francis GJ, Langton SR, Goodwin CS, Blincow ED. Rapid urease test in the management of *Campylobacter pyloridis*-associated gastritis. *Am J Gastroenterol* 1987; 82: 200–10.
- Das SS, Bain LA, Karim QN, Coelho LG, Baron JH. Rapid diagnosis of *Campylobacter pyloridis* infection. *J Clin Pathol* 1987; 40: 701–2.
- Hazell SL, Borody TJ, Gal A, Lee A. *Campylobacter pyloridis* gastritis. I. Detection of urease as a marker of bacterial colonisation and gastritis. *Am J Gastroenterol* 1987; 82: 292–6.
- Vaira D, Holton J, Cairns S, Falzon M, Salmon P. Four hour rapid urease test (RUT) for detecting *Campylobacter pylori*: is it reliable enough to start treatment? *J Clin Pathol* 1988; 41: 355–6.
- Vaira D, Holton J, Cairns S, et al. Urease tests for *Campylobacter pylori*: care in interpretation. *J Clin Pathol* 1988; 41: 812–3.
- Arvind AS, Cook RS, Tabaqchali S, Farthing MJG. One minute endoscopy room test for *Campylobacter pylori*. *Lancet* 1988; i: 704.
- Skirrow MB. *Campylobacter enteritis*: a 'new' disease. *B MJ* 1977; ii: 9–11.
- Mobley HLT, Cortesia MJ, Rosenthal LE, Jones BD. Characterization of urease from *Campylobacter pylori*. *J Clin Microbiol* 1988; 26: 831–6.
- Fleiss JL. The comparison of proportions from many samples. In: *Statistical methods for rates and proportions*. Chichester: Wiley, 1973: 92–108.
- Raisanen S, Sodervik H. Colonisation of gastric lesions by urease-positive bacteria. *Am J Clin Pathol* 1988; 90: 749.
- Wyatt JJ, Primrose J, Dixon MF. Distribution of *Campylobacter pylori* in gastric biopsies. *J Pathol* 1988; 155: 350A.
- Kalder J, Tee W, McCarthy P, Watson J, Dwyer B. Immune response to *Campylobacter pylori* in patients with peptic ulceration. *Lancet* 1985; i: 921.
- Rathbone BJ, Wyatt JJ, Worsley BW, et al. Systemic and local antibody responses to gastric *Campylobacter pylori* in non-ulcer dyspepsia. *Gut* 1986; 27: 642–7.
- Booth L, Holdstock G, MacBride H, et al. Clinical importance of *Campylobacter pyloridis* and associated serum IgG and IgA antibody responses in patients undergoing upper gastrointestinal endoscopy. *J Clin Pathol* 1986; 39: 215–9.
- McNulty CAM, Dent JC, Uff JS, Gear MWL, Wilkinson SP. Detection of *Campylobacter pylori* by the biopsy urease test: an assessment in 1445 patients. *Gut* 1989; 30: 1058–62.