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Detection of *Campylobacter pylori* by the biopsy urease test: an assessment in 1445 patients

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SUMMARY The presence of *C pylori* infection was determined in 1445 patients undergoing upper gastrointestinal endoscopy over a 12 month period. The presence of *C pylori* was detected in gastric mucosal biopsy specimens by the biopsy urease test, microscopy (Gram stained smears and histology) and culture. Two media were used for the biopsy urease test: Christensen's urea broth (for the first 600 patients) and the Christensen's urea broth modified by increasing the concentration of phenol red and omitting the nutrients, glucose and peptone (for the remaining patients). Both the Christensen's urea broth and modified urea broth were almost 100% specific when compared with detection of *C pylori* by Gram, culture and histopathology. The modified broth was more sensitive (96% sensitivity compared with culture) than the Christensen's broth (92% sensitivity) but this difference was not statistically significant. The modified broth gave significantly more positive results (58%) in less than 30 minutes than the Christensen's broth (48%). Seventy four per cent of positive results were available in less than two hours. Specimens from patients with extensive *C pylori* infection gave more rapid results: 86% of specimens that yielded a profuse growth of *C pylori* and 76% that contained numerous organisms on histological sections had a positive urease test in less than one hour. There was no significant difference between the specificity and sensitivity of our modified urea broth and the other modified broths described in the literature. This test is a cheap and rapid alternative to the diagnosis of *C pylori* by Gram stained smears or culture.

There is now strong evidence that *Campylobacter pylori* is an important aetiological factor in gastritis and peptic ulceration.^{1,2} Initially this infection was diagnosed by microscopy of tissue smears or sections, or by prolonged microaerobic culture. These techniques are relatively cumbersome and slow, and a more simple, rapid diagnostic method would seem desirable. Langenberg *et al* first reported that *C pylori* produced large amounts of urease³; this character was then used for the rapid identification of *C pylori* cultures. Our initial observation that there was enough preformed urease produced by the organisms to detect *C pylori* in biopsy specimens led to a small pilot study that confirmed the ease and

value of this test in the rapid diagnosis of this infection.⁴ Since this first report many workers worldwide have modified the biopsy urease test (Table 1). Hazell *et al*⁵ have reported a series of 376 patients, but many of the series have been small and the reports not detailed; this is especially so for those describing modified rapid tests.⁶⁻⁹ This is the first full report of the biopsy urease test in a very large series of patients (1445) attending for upper gastrointestinal endoscopy.

Methods

PATIENTS

All patients attending endoscopy for the investigation of upper gastrointestinal symptoms (mainly dyspepsia) or follow up after treatment for peptic

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Table 1 Value of the different biopsy urease tests

Author	Test name§	n	Sensitivity	Specificity	Speed
Marshall <i>et al</i> ^a	CLO Test	141	98%		75% 20 m
Hazell <i>et al</i> ^c	Microtitre BUT‡	376	91%	100%	75% 1 h
Vaira <i>et al</i> ^b	4 hour RUT*	256	89%	100%	100% 4 h
Arvind <i>et al</i> ^b	1 minute test‡	40	90%	100%	100% 1 m
McNulty <i>et al</i> [†]	Christensen's‡	597	92%	99.4%	61% 1 h 71% 2 h
McNulty <i>et al</i> [†]	Modified BUT‡	847	96%	100%	68% 1 h 74% 2 h

*Incubated at 37°C; †Present series; ‡Compared with culture; §CLO – *Campylobacter* like organisms, BUT – biopsy urease test, RUT – rapid urease test; ||% +ve at stated time, m – minutes, h – hours.

ulceration from June 1986 to June 1987 were considered for the study. Patients with any contraindication to gastric biopsies were excluded. The study was approved by Gloucestershire Royal Hospital ethical committee. Patients gave informed consent for endoscopy and biopsy.

BIOPSY SPECIMENS

Four gastric biopsy specimens were taken from within 5 cm of the pylorus. Two specimens for histology were placed in 10% formol saline. Two specimens for microbiology were placed on the glass near the top of a small sterile bottle containing 100 µl sterile saline (to maintain humidity). The specimens were not placed in the saline, as immersion led to poor tissue smear preparations.

HISTOLOGY

Formalin fixed biopsy specimens were processed to paraffin in the usual way. Sections were stained by haematoxylin and eosin (H&E), periodic-acid Schiff (PAS) and the half-Gram method.¹⁰ Biopsy specimens were assessed by a histopathologist without knowledge of the patient's details. The presence or absence of *Campylobacter* like organisms was noted on the half Gram stained sections, and the number of organisms scored from zero to three. Where 0=no bacteria seen; 1=occasional bacteria present, not seen in every high power field; 2=moderate numbers seen; 3=numerous bacteria in all fields.

MICROBIOLOGY

Specimens were processed within two hours of collection. *C. pylori* was sought by microscopy of Gram stained tissue smears, culture, and the biopsy

urease test. Each specimen was smeared with a swabstick onto a sterile glass slide, across two culture plates (see below) and finally crushed with the swab in 0.5 ml urea medium.

MICROSCOPY

The tissue smears were air dried, heat fixed, and stained by Gram's method using dilute carbol fuchsin as counter stain. Smears were examined for five minutes with a ×100 oil immersion lens for the characteristic Gram negative spiral organisms.

CULTURE

The culture media used were chocolate agar (10% horse blood, Oxoid Columbia agar base CM331) and *Campylobacter* selective agar (7% lysed horse blood in Columbia agar base (CM331) with trimethoprim 5 mg/l, vancomycin 10 mg/l, cefsulodin 5 mg/l, and amphotericin 5 mg/l). Plates were incubated micro-aerobically (6% O₂; partial evacuation to 500 mmHg and gas replacement with 10% H₂, 10% CO₂, 80% N₂) at 37°C and examined after three, five, and seven days. Gram-negative spiral organisms with characteristic colonial appearance and a rapid positive urease test were deemed to be *C. pylori*.

BIOPSY UREASE TEST

The test detects the presence of preformed urease produced by *C. pylori* in biopsy specimens; growth of the organisms is not required. The urease present hydrolyses urea in the broth with the production of ammonium ions which raise the pH. This pH change is detected by the phenol red indicator, which changes colour from yellow/brown at pH 6.8 to pink at pH 8.4. The swab stick used to prepare the tissue smears and inoculate the plates was used to crush the two specimens together and broken off into 0.5 ml of urea broth. The biopsy urease tests were left at room temperature in the laboratory where they could be examined regularly for the first two to six hours (a maximum of two hours for specimens received in the afternoon), and at 18 or 24 hours. A colour change from yellow/brown to pink on the swab or in the broth was considered positive. The time of the colour change was noted.

After testing 600 patients the formula of the Christensen's urea broth (urea 20 g/l, phenol red 0.012 g/l, KH₂PO₄ 2 g/l, NaCl 5 g/l, peptone 1 g/l, glucose 10 g/l¹¹) was modified. Nutrients in Christensen's broth allow the growth of some aerobic urease producing organisms that can lead to false positive results, so in the modified broth the peptone and glucose were excluded. In an attempt to produce a more rapid result and increase the test sensitivity the concentration of the phenol red was increased from 0.012 to 0.04 g/l.

Results

Specimens were inadequate for histological assessment in 14 of the 1445 patients. *C pylori* was detected by Gram, culture, or histopathology in 628 (43.5%) patients. There was concordance between the detection of *C pylori* by histology and microbiology (Gram or culture) in 1342 (94%) of patients; each method failed to detect 7% of positives. Thirty four of the 46 not detected by histology yielded a scanty growth only and 20 of the 42 not detected by microbiology had scanty organisms only seen on histology.

BIOPSY UREASE TESTS

When the results from the two urease tests were combined, the test was almost 100% specific and 95% sensitive with only three false positives and 32 false negatives when compared with culture.

CHRISTENSEN'S BROTH

This broth was used in 596 patients – specimens from four patients were excluded as transport was delayed. *C pylori* was detected in 258 patients by Gram, culture or histology (Table 2). When compared with Gram and culture there were only two apparent false positives (specificity 99.4%); the test was negative in 19 specimens that yielded *C pylori* on culture (sensitivity 92%). When compared with the detection of *C pylori* by all three methods (Gram, culture and histology) the test had a specificity of 100% and sensitivity of 85% (38 false negatives) – Table 2. Eight of the false negatives were positive only by microbiology and 18 were positive only by histology; in half of these organisms were scanty.

MODIFIED BROTH

C pylori was detected by Gram, culture, or histology in 370 of 849 patient specimens assessed with the modified broth (Table 2). The modified broth was highly specific. There was one test that was positive (20 minutes after inoculation) that was negative by Gram and culture; this patient's specimen yielded a profuse growth of a urease positive *Staphylococcus aureus*. When compared with the detection of *C pylori* by all three methods (Gram, culture, and histology) the modified broth had a specificity of almost 100% (one false positive mentioned above) and sensitivity of 90% (37 false negatives). Twenty three of these false negatives were detected only by histology and seven only by microbiology. The modified broth was slightly more sensitive than the Christensen's broth with only 13 false-negatives (sensitivity 96%) compared to culture but this difference was not statistically significant ($\chi^2=2.24$), Table 3.

Table 2 Detection of *Campylobacter pylori* by the different methods

	Microbiology				Microbiology or Histology			
	Gram		Culture		Histology*		or Histology	
	+	-	+	-	+	-	+	-
Christensen's (596)								
Biopsy urease +ve (220)	218	2	218	2	204	14	220	0
Biopsy urease -ve (376)	16	360	19	357	30	341	38	338
Modified (849)								
Biopsy urease +ve (334)	333	1	333	1	312	18	333	1
Biopsy urease -ve (515)	14	501	13	502	30	482	37	479

*1431 specimens assessed by histology.

Table 3 Specificity and sensitivity of the Christensen's and modified urea broths compared with detection by Gram or culture, and Gram, culture, or histology

	Christensen's		Modified	
	Gram/ culture	Gram/ culture/ histology	Gram/ culture	Gram/ culture/ histology
Specificity	99.4%	100%	99.8%	99.8%
Sensitivity	92%	85%	96%	90%
Predictive value of positive	100%	100%	99.7%	99.7%
Predictive value of negative	95%	89%	97%	93%

Table 4 Times at which positive urea broth tests were recorded

Time positive	Number of tests +ve (cumulative percentage)	
	Christensen's (n=220)	Modified (n=334)
<30 m	105 (48)	195 (58)*
<1 h	134 (61)	228 (68)
<2 h	157 (71)	247 (74)
<4 h	177 (80)	260 (78)
>4 h	43	73

* $\chi^2=6.06$, $p<0.01$.

SPEED OF THE UREA BROTH (Table 4)

The modified broth produced significantly more positive results in the first hour than the Christensen's broth ($\chi^2=6.06$), but there was no statistically significant difference between the speed of the tests after this time. Seventy one per cent of tests were positive with the Christensen's and 74% with the modified urea broth in less than two hours.

Cultures which yielded a profuse growth or in

which large numbers of organisms were seen by histology (score of 3) gave more rapid biopsy urease results. Seventy five specimens tested with the modified broth yielded a profuse growth of *C pylori*; 65 (87%) gave positive modified urease results in less than 30 minutes, 71 (95%) in one hour. By contrast, only 15 (19%) of 80 specimens yielding a scanty growth gave a positive modified urea broth test in less than 30 minutes ($\chi^2=71.5$, $p<0.01$). Combining the results from both the Christensen's and modified tests 100 of 131 (76%) specimens yielding a profuse growth compared to 29 of 165 (18%) yielding a scanty growth gave a positive test in less than 30 minutes ($\chi^2=103$, $p<0.001$).

Discussion

There was disagreement in the diagnosis of *C pylori* by histology or microbiology in only 88 (6%) of the 1431 specimens in which histology was available. This small lack of concordance between microbiology and histology is probably due to sampling variation. *C pylori* infection can be patchy and because the biopsy specimens are small, organisms may be present in one specimen and not another. Ideally to detect all positives – for example, in a treatment trial, it is necessary to perform microbiology and histology on all specimens. The use of both departments is more time consuming and in most endoscopy departments the use of the biopsy urease test alone will be sensitive enough and much more convenient.

Both the Christensen's and modified broths were extremely specific so no patients will be treated unnecessarily with antimicrobial agents as a result of this test. Compared with the other diagnostic methods both tests were also very sensitive (sensitivities: Christensen's 92%, and modified 96%); although the modified broth appeared more sensitive than the Christensen's broth this was not statistically significant. Both tests had very good predictive value for positive and negative specimens (Table 3) and therefore could be used to replace culture or microscopy for the detection of *C pylori*. Although the noninvasive urea breath tests¹² are becoming more widely available, we believe that clinicians will continue to perform endoscopy and biopsy for the investigation of upper gastrointestinal symptoms. The biopsy urease tests are very easy to prepare, perform, and to read and have the great advantage that they can be used in the endoscopy unit, yielding a result before the patient leaves the department. These factors will achieve a cost saving as an outpatient appointment may be avoided and the use of the diagnostic departments will be decreased. Since the modified test is a more simple formula, is slightly more sensitive and produces more rapid

results we would suggest its use in preference to the Christensen's broth.

When different urease tests are compared it is important to note the characteristics of the patient population and the definition of *C pylori* positivity, as these will alter the reported predictive value of the test. The biopsy urease tests described so far all have a sensitivity of near 90% or above (Table 1) and the choice of test will be determined by the time within which the result is required and the ease of preparing and performing it. The CLO test as described by Marshall *et al* has the highest sensitivity.⁶ This test is incubated at 30°C which is nearer the optimum temperature for urease (45°C),¹³ so fewer organisms may be needed to produce a positive result. The speed of all the tests would be improved by incubation at this optimum temperature, which could be attained by the use of a small thermostatically controlled waterbath. The high sensitivity of the CLO test has been confirmed by Morris *et al*,⁷ however, Vaira *et al* found that in their hands the CLO test had only a 58% sensitivity.¹⁴ Our modified urea test had a good sensitivity, and this has been confirmed by Vaira *et al*¹⁴ who found that the 2% urea broth had a 94% sensitivity. The CLO test is a commercial product and is therefore the most expensive test (£2/test from Bripharm Ltd, UK), but it can be performed with ease without the use of a microbiology department. In contrast the 2% urea broth test can be made by the majority of microbiology departments, for use in endoscopy, and cost less than 5p/test.

The small increase in sensitivity with the modified broth is probably because of the increased concentration of phenol red which makes the colour change easier to detect. The removal of nutrients only effects the false positivity and has no effect on the sensitivity. Most specimens (nine of 13) that gave a negative modified biopsy urease result and were positive by culture yielded a scanty growth and thus contained insufficient organisms to produce the pH change required. In our method we prepared a tissue smear and used the specimens for culture before inoculation of the test. This decreases the number of organisms inoculated and decreases the sensitivity and speed. Deltenre *et al* have found that crushing the specimens in the urea broth at the time of endoscopy, and avoiding the use of transport media increased the sensitivity and speed.¹⁵

Although false positives are a potential problem with these tests we have very few even in the nutrient containing Christensen's broth. There are several reasons for the high specificity of all the urease tests: contaminating bacteria are usually present in low numbers compared with *C pylori*; no other organism produces urease with such high affinity or rate

of activity,¹³ and the specimens are left at room temperature so preventing the rapid growth of most organisms.

Our modified broth produced more positive results in the first 30 minutes than the Christensen's broth. The modified broth is as fast as Hazell's microtitre method⁵ but not as fast as the CLO test reported by Marshall⁶ and Morris,⁷ or the rapid high concentration urea tests.^{8,9} With both our tests there was a correlation between the degree of infection with *C pylori* and the speed of the test, this has also been noted by Hazell and Morris.^{5,7} This difference in speed was not seen by Marshall or with the four hour RUT. If in the future it became evident that the severity of infection was important it would be useful to have a test which would differentiate scanty from profuse colonisation.

Both the high concentration, rapid urease tests described recently have been assessed only on a small number of specimens (90 for the 6%,¹⁴ 40 for the 10% urea⁸) and therefore the results should be interpreted with some caution. The ideal concentration of substrate in an enzymic reaction is usually 50 to 100 times the Km value of the enzyme (a measure of affinity of an enzyme for its substrate), so that the substrate is in a large excess, and the reaction rate is dependent only on the enzyme activity. *C pylori* urease has a Km of 0.32 g/l (8 mM); the ideal concentration of urea would therefore be about 16–32 g/l (1.6–3.2%). Too much substrate may result in inhibition of an enzyme. The 6% and 10% solutions therefore probably do not increase sensitivity and may even inhibit the urease activity. The rapid results are probably the result of the absence of buffer in these two tests, but this may decrease the stability and thus specificity. Arvind *et al* acknowledge the instability and indicate that their 10% urea broth should be freshly prepared before use. The advantage of the rapid results produced by these tests may outweigh the disadvantage of mixing the reagents in the endoscopy unit; this will depend on endoscopy staffing. Much larger studies are needed to assess the shelf life and predictive value of these more rapid tests.

Changing the pH of the broth so it is closer to the ideal pH for urease activity (pH 8.2)¹³ might increase the sensitivity and speed of the test. This change will necessitate the use of an indicator with a higher pH-range – for example, m-cresol purple (pH range 7.4–9.0) or thymol blue (pH range 8.0–9.6), and a buffer with a higher pK.

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