SENSITIVITY AND SPECIFICITY OF AN IN-HOUSE RAPID UREASE TEST FOR DETECTING *HELICOBACTER PYLORI* INFECTION ON GASTRIC BIOPSY

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Abstract. We developed an in-house rapid urease test (iRUT) and evaluated the efficacy and the agreement of the iRUT and the cRUT compared with culture and histology for the detection of *H. pylori* infection. Five iRUT media were tested with *H. pylori* isolates and other bacteria. The most suitable iRUT medium was further evaluated for detection of *H. pylori* infection. Gastric biopsies from 120 patients were diagnosed by culture, iRUT, cRUT and histology. The results of the iRUT and cRUT were read at 30 minutes, 1 hour and up to 24 hours. A true positive result was either the culture or both the RUT (cRUT or iRUT) and the histological examination being positive. The sensitivity and specificity of the iRUT result at 30 minutes, 1 hour and up to 24 hours were 77.1% and 100%, 77.6% and 100%, and 94.1% and 94.2%, respectively. Values for the same parameters of cRUT were 87.5% and 100%, 89.8% and 100%, and 100% and 94.2%, respectively. The agreement between the iRUT and cRUT was very good (kappa values ≥ 0.82). Our results indicate that the iRUT is a sensitive, specific and cost effective test. It can be appropriately applied for detecting *H. pylori* infection in gastric biopsy specimens.

INTRODUCTION

Helicobacter pylori is a gram-negative curved-to-spiral microaerobic bacteria, considered an important etiological agent in the development of gastritis, peptic ulcers and gastric carcinoma (Ansorg *et al*, 1991; Parsonnet, 1994; Logan and Walker, 2001). At present, there are several techniques available for the detection of *H. pylori,* including bacterial culture, histological examination, serological testing, a rapid urease test (RUT), a urea breath test (UBT) and polymerase chain reaction (Fabre *et al*, 1994; Heatley, 1995; Kisa *et al*, 2002). RUT is an attractive diagnostic method because it is rapid, sensitive, specific and requires only visual interpretation (Kawanishi *et al*, 1995; Onders, 1997; Said *et al*, 2004). It can give a presumptive result which expedites therapeutic decision making (Onders, 1997; Kuo *et al*, 2002; Lim *et al*, 2004; Morio *et al*, 2004). However, the unit cost for cRUT is relatively expensive for routine work in developing countries, such as Thailand.

The development of an iRUT has been reported (Goldie *et al*, 1989; Forman *et al*, 1994; Pajares-Garcia, 1998; Adesanya *et al*, 2002), but some iRUTs have had disadvantages such as containing sodium azide, which is potentially toxic (Hazell *et al*, 1987). Some iRUTs have been insensitive, time consuming and have false positive results, especially when long incubation times were used to increase the sensitivity (Das *et al*, 1987; Ho *et al*, 1996).

There are several types of iRUT including buffered and unbuffered urea media, and liquid and semisolid media (Vaira *et al*, 1988; Thillainayagam *et al*, 1991; Kuo *et al*, 2002; Montes *et al*, 2003). The results for each iRUT are different (Goldie *et al*, 1989; Forman *et al*, 1994; Pajares-Garcia, 1998; Adesanya *et al*, 2002). We developed and tested (*in vitro*) five iRUT formulae and sought one that would allow easy interpretation after a short incubation period without false positive results due to other urease positive organisms. We tested the sen-

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sitivity and specificity of iRUT on gastric biopsies from dyspeptic patients by comparing with standard culture, histological examination and cRUT. The stability of the medium and unit cost were also determined.

METHODS

Patients and endoscopy

One hundred twenty consecutive patients with dyspeptic symptoms who underwent upper gastrointestinal endoscopy were included in this study. They were recruited from the Endoscopy Unit of Srinagarind Hospital, Faculty of Medicine, Khon Kaen University between February 2002 and February 2004. The subjects were diagnosed as non-ulcer dyspepsia (NUD), peptic ulcer dyspepsia (PUD), gastric carcinoma (GCA) and other gastrointestinal diseases (GERD, duodenitis, etc).

We excluded patients who had antibiotic therapy, bismuth treatment, proton pump inhibitors, or H2-blockers within the previous month. Informed consent was obtained from each patient before being included in the study.

Biopsy specimens

Four antral and four corpus biopsy specimens were obtained from each patient and divided into four parts. Both antral and corpus specimens were used for cultures, a commercial rapid urease test (cRUT), an in-house rapid urease test (iRUT) and histological examination.

Culture

The culture was performed according to Hazell *et al* (1989) with modification. Briefly, each antral and corpus specimen was immediately placed into transport media and brought to the laboratory within 2 hours, and stored under cold conditions. The biopsy specimens were homogenized in 200 μl of normal saline and cultured on 7% human blood agar (Difco, Detroit, Michigan, USA) containing the supplement SR147 (5 mg/l trimethoprim +10 mg/l vancomycin + 5 mg/l amphotericin B + 5 mg/l cefsulodin, SR147, OXOID). The plates were incubated at 37ºC under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) and were examined after 4 and 7 days of incubation. Characteristic colonies of *H. pylori* were confirmed by Gram staining, oxidase, catalase and urease tests.

Commercial rapid urease test (RUT, Pronto Dry test)

The RUT was performed according to the manufacturer's instructions (Medical Instruments Corporation, Solothurn, Switzerland). Briefly, one antral and one corpus specimen were directly inoculated onto the cRUT agar gel. The results were observed and recorded at 24 hours. a positive was indicated when the color changed from yellow to pink.

Histological examination

One antral and one corpus biopsy were fixed in 10% buffered formalin, processed, then embedded in paraffin. Four slices 3-4 μm thick were stained with modified Warthin-Starry stain for identification of *H. pylori* (Cohen and Laine, 1997; Li *et al*, 2004). The presence of spiral organisms on any of the slides was considered positive for *H. pylori.*

In-house rapid urease test (iRUT)

Organisms. *Proteus mirabilis* (a strong urease producer), *Pseudomonas aeruginosa* (a weak urease producer), and *Escherichia coli* (a urease negative organism) were grown on blood agar under atmospheric conditions for 24 hours. Four isolates of *H. pylori* were grown on blood agar under microaerophilic conditions for 4 days. The concentration of *H.pylori* were adjusted to 10^8 organisms/ml in 1 % proteose peptone water at McFarland standard no. 3 (Xia *et al*, 1994) and the other bacteria at McFarland standard no. 0.5 (National Committee for Clinical-Laboratory Standards, 2002). Ten fold serial dilutions were prepared ranging from 10^7 to 10^2 cells/ml. Ten microliters of each dilution was then used for the iRUT.

Media. The following 5 media were tested: Formula I was comprised of urea 20 g/l, KH_2PO_4 2 g/l, phenol red 0.012 g/l, NaCl 5 g/l, peptone 1 g/l, glucose 10 g/l and agar 4 g/l (Modified Christensen urease test). Formula II was comprised of urea 20 g/l, $NaH_2PO_4.H_2O$ 1.4 g/l, phenol red 0.012 g/l and agar 4 g/l (Buffered medium). Formula III was comprised of urea 20 g/l, phenol red 0.012 g/l and agar 4 g/l.(Unbuffered medium). Formula IV was comprised of urea 20 g/l, $NaH_2PO_4.H_2O$ 1.4 g/l, phenol red 0.04 g/l

and agar 4 g/l (Buffered medium). Formula V was comprised of urea 20 g/l, phenol red 0.04 g/l and agar 4 g/l (Unbuffered medium).

After preparation, 2 ml of each medium was aseptically dispensed into a steriled 24 well plate, wrapping with aluminium foil and stored at 4°C until used.

Test for optimal medium. The five formula media were tested for the optimal iRUT medium used. One hundred microliters of each bacterial suspension was inoculated into each well of medium. The results were observed and recorded after 30 minutes, and at 1, 2, 4 and 24 hours. The grading criteria were interpreted according to the following criteria: 1) negative, when the medium had no color (yellow); 2) positive 1+ , when the color changed from yellow to pale pink; 3) positive 2+, when the color changed from yellow to pink; 4) positive 3+ , when the color changed from yellow to red and; 5) positive 4+, when the color changed from yellow to deep red or purple.

Test for specimens. One antral and one corpus specimen were each directly inoculated onto an iRUT. The results were observed and recorded, the same as the cRUT.

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the iRUT were evaluated by comparing with culture, histological examination and the cRUT. While the same parameters for the cRUT were evaluated by comparing with culture, histological examination and the iRUT.

The criteria for a true positive *H. pylori* result was considered as having a positive result

Table 1
In intro IRUT testing for *H. pylori, P. mirabilis, Ps. aerginosa* and *E. coli.*

Organisms and Reaction time	Reaction[a] with the following organisms/ml					
	10^7	10^6	10^5	10^4	10^3	10^2
H. pylori (isolate 1)						
≤ 30 minutes	3+	2+	-	-	-	-
≤ 1 hour	3+	2+	-	-	-	-
≤ 4 hours	4+	2+	1+	-	-	-
≤ 24 hours	4+	2+	1+	-	-	-
H. pylori (isolate 2)						
≤ 30 minutes	3+	1+	-	-	-	-
≤ 1 hour	3+	1+	-	-	-	-
≤ 4 hours	4+	3+	-	-	-	-
≤ 24 hours	4+	3+	-	-	-	-
H. pylori (isolate 3)						
≤ 30 minutes	4+	2+	-	-	-	-
≤ 1 hour	4+	3+	-	-	-	-
≤ 4 hours	4+	4+	-	-	-	-
≤ 24 hours	4+	4+	1+	-	-	-
H. pylori (isolate 4)						
≤ 30 minutes	2+	1+	-	-	-	-
≤ 1 hour	4+	1+	-	-	-	-
≤ 4 hours	4+	2+	-	-	-	-
≤ 24 hours	4+	2+	-	-	-	-
P. mirabilis, E. coli, *Ps. aeruginosa*						
≤ 24 hours	-	-	-	-	-	-

[a]Grading of reaction: - = no color change, 1+ = pale pink, 2+ = pink, 3+ = red, 4+ = deep red to purple

Table 2
Numbers and percentages of 120 *H. pylori* infections detected by culture, histology, cRUT and iRUT.

Culture	Histology	cRUT	iRUT	Total No. (%)	Evaluation of infection[a]
+	+	+	+	39 (32.5)	TP
+	+	+	-	3 (2.5)	TP
-	+	+	+	9 (7.5)	TP
-	-	+	+	4 (3.3)	FP
-	+	-	-	25 (20.8)	FP
-	-	-	-	40 (33.3)	TN
	Total			120 (100)	51 (42.5%)

TP = True positive, TN = True negative, FP = False positive
[a] = TP, culture positive on both urease and histological examination positive

Table 3
Sensitivity, specificity, positive and negative predictive values and accuracy of the iRUT compared with the gold standard[c].

Time	Sensitivity (%)	Specificity (%)	PPV[a] (%)	NPV[b] (%)	Accuracy (%)
≤ 30 minutes	77.1 (37/48)	100 (72/72)	100 (37/37)	86.7 (72/83)	90.8 (109/120)
≤1 hour	77.6 (38/49)	100 (71/71)	100 (38/38)	86.6 (71/82)	90.8 (109/120)
≤ 2 hours	79.6 (39/49)	100 (71/71)	100 (39/39)	87.6 (71/81)	91.6 (110/120)
≤ 24 hours	94.1 (48/51)	94.2 (65/69)	92.3 (48/52)	95.6 (65/68)	94.1 (113/120)

[a]Positive predictive value; [b]Negative predictive value; [c]The criteria for the gold standard were positive culture and/or positive histology and cRUT.

on either the culture or RUT and histological examination (Pajares-Garcia, 1998; Liao *et al*, 2003). The degree of agreement between the commercial and iRUT was also analyzed by a Kappa statistic (Landis *et al*, 1977).

RESULTS

We selected the medium that gave a positive reaction in the shortest time, at the lowest concentration of *H. pylori,* while giving a negative reaction with other organisms. The buffered medium formula II (urea 20 g/l, NaH_2PO_4 1.4 g/l, phenol red 0.012 g/l and agar 4 g/l) was the optimal medium, since the positive results were observed within 30 minutes when tested with 4 *H. pylori* isolates at 10^5-10^6 organisms/ml or 10^3-10^4 cells when loaded in 10 μl (Table 1). Moreover, it was easy to differentiate between a weakly positive or a weakly negative result compared with the other formulae.

The medium was further applied to the gastric biopsies from 120 dyspeptic patients. *H. pylori* was detected by culture, histological examination, cRUT and iRUT in 42 (35%), 76 (63.3%), 55 (66%) and 52 (43.3%), respectively. Regarding the true positive test criteria, *H. pylori* infection was found in 42.5% (Table 2). The sensitivity and specificity for the iRUT were 77.1% and 100%, 77.6 and 100%, 79.6% and 100%, and 94.1% and 94.2 % when tested at ≤ 30 minutes, ≤1 hour, ≤2 hours and ≤24 hours, respectively (Table 3). Values of the same parameters of cRUT have been shown in Table 4.

A false positive reaction was not found for either the iRUT or the cRUT at ≤ 30 minutes or ≤1 hour but was found in 4 of 120 specimens (3.33 %) at 24 hours. Four of them were positive by both the iRUT and the cRUT, but nega-

Table 4
Sensitivity, specificity, positive and negative predictive values, and accuracy of cRUT with the gold standard[c].

Time	Sensitivity (%)	Specificity (%)	PPV[a] (%)	NPV[b] (%)	Accuracy (%)
≤ 30 minutes	87.5 (42/48)	100 (72/72)	100 (42/42)	92.3 (72/78)	95 (114/120)
≤1 hour	89.8 (44/49)	100 (71/71)	100 (44/44)	93.4 (71/76)	95.8 (115/120)
≤ 2 hours	92 (46/50)	100 (70/70)	100 (46/46)	94.6 (70/74)	96.7 (116/120)
≤ 24 hours	100 (51/51)	94.2 (65/69)	92.7 (51/55)	100 (65/65)	96.7 (116/120)

[a]Positive predictive value; [b]Negative predictive value; [c]The criteria for the gold standard must be positive culture and/or positive histology and the iRUT.

Table 5
Percentages of positive reactions by time interval for iRUT and cRUT.

RUT	Percentage of true positives by time interval[c]			
	≤ 30 min	> 30 min - 1 hr	> 1 hr - 2 hr	> 2 hr - 24 hr
In house (N = 48)	77.1 (37/48)	2.1 (1/48)	2.1 (1/48)	18.7 (9/48)
Commercial (N = 51)	82.4 (42/51)	3.9 (2/51)	3.9 (2/51)	9.8 (5/51)

[c]True positives for both cRUT and iRUT compared with gold standard criteria

Table 6
Agreement between iRUT and cRUT, culture and histological examination.

Time	Kappa value [a]		
	cRUT	Culture	Histology
≤ 30 min	0.88	0.75	0.41
≤ 1 hr	0.84	0.77	0.42
≤ 2 hr	0.82	0.75	0.46
≤ 24 hrs	0.95	0.78	0.48

Kappa value [a] < 0.0, poor; 0.00 - 0.20, slight; 0.21 - 0.40, fair; 0.41 - 0.60, moderate; 0.61 - 0.80, substantial; 0.81 - 1.00, almost perfect

tive by culture and histology. The positive reactions according to time for iRUT and cRUT are shown in Table 5. The agreement between iRUT and cRUT, culture and histology analyzed by the kappa statistic is shown in Table 6. The agreement was very good (kappa > 0.81) between iRUT and cRUT. The medium was stable for up to 5 months after being stored in a refrigerator and protected from light (data not shown).

DISCUSSION

Dyspeptic patients infected with *Helicobacter pylori* are generally investigated by the physician during endoscopy. Detection of *H. pylori* infection on gastric biopsy specimens commonly uses RUT because the results can be interpreted easily, rapidly and can give a result before patient is discharged from the endoscope room (Kuo *et al*, 2002; Lim *et al*, 2004). Some results for cRUT, such as Pronto Dry and CLO, were compared. The findings show that the Pronto Dry test has a quicker positive reaction time and the positive color change is more distinct (Said *et al*, 2004). Although highly sensitivie and specific, the cost is relatively expensive. A rapid and economical RUT is needed to give results before the patient leaves the endoscope unit. Some researchers developed an iRUT with a sensitivity and specificity comparable to histological examination of between 65% and 100% at 4 hours, and 83% to 100% at 24 hours, respectively (Cifuentes *et al*, 2002). Our study was designed to evaluate the performance of an iRUT for the diagnosis of *H. pylori* infection, using culture, histology and cRUT (Pronto Dry) as

the gold standard.

For the best medium for the iRUT, we compared buffered medium, unbuffered medium and modified Christensen urease with different amounts of phenol red. The unbuffered mediums immediately changed color when tested with a high concentration of bacteria, but it was difficult to differentiate between a negative and a weakly positive reaction. There was also poor specificity, with false positives occuring in less than 24 hours (data not shown).

Of the three buffered media, we chose formula II as the most suitable medium because it was easy to determine a positive result within 1 hour. Color change could be observed when the organisms were at a low concentration of approximately 10^3-10^4cells (10^5-10^6 organisms/ml). This is comparable to other investigations where a positive RUT required the presence of approximately10^5 to 10^7 organisms/ml (Vaira ***et al***, 1988; Goldie ***et al***, 1989).

Some researchers have used liquid urease for the detection of *H. pylori*. The results showed sensitivity and specificity of 91% and 88%, respectively (Montes ***et al***, 2003). A previous report showed RUT broth was unstable, required the addition of phenol red indicator before use and required many more organisms ($\geq 1 \times 10^6$) to show a color change (Thillainayagam ***et al***, 1991).

There were 3 (2.5%) false negative specimens on the iRUT (Table 2). However, the specificity of the iRUT was excellent, there were no false positive results found in ≤1 hour and only 3.3% (4/120) were seen on iRUT and cRUTs at 24 hours (Table 2). Most iRUT and cRUT showed positive results within 30 minutes in 77.1% and 87.5%, respectively. The sensitivity and specificity of iRUT increased to 94.1% and 100% at 24 hours, similar to previous studies. Previous researchers have reported that RUT increased in sensitivity and reduced in specificity with longer incubation times (Adesanya ***et al***, 2002; Cifuentes ***et al***, 2002; Viiala ***et al***, 2002; Lim ***et al***, 2004).

Several factors may affect the results on RUT, such as the amount of urea, the incubation temperature, the indicator used in the medium and the number of biopsies used in a RUT. In our study, we modified the formula of iRUT based on the Christensen urease test that used 2% urea and 0.12% phenol red as an indicator. It has been suggested that increasing the concentration of urea to 3% may increase the sensitivity of the iRUT. Mobley ***et al*** (1988) reported the optimal temperature of urease activity for *H. pylori* is 45ºC. Laine ***et al*** (1996) reported incubation of CLO test at 37ºC hastened the time to a positive test and specificity was not influenced by warming. Thus, increasing the incubation temperature of iRUT from 37ºC to 45ºC may accelerate the color change.

The number of biopsies used in a RUT affects the results because some reseachers have shown that taking two biopsies instead of one has resulted in an earlier positive RUT result (Lim ***et al***, 2004). Therefore, we used one antral and one corpus biopsy specimen in our RUT test.

McNulty ***et al*** (1989) suggested that adjustment of the pH of the medium and the use of an indicator with a pH range closer to ideal pH of urease activity (pH 8.2) may increase the sensitivity and decrease the time to develop a positive result. The use of an indicator with a higher pH range than phenol red (pH range 6.4-8.2), such as 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 should be evaluated further (McNulty ***et al***, 1989). However, phenol red at a pH of 6.8 is generally used as an indicator (Adesanya ***et al***, 2002).

We found the agreement between the iRUT and cRUTs was “very good” or “almost perfect” (kappa > 0.82) whereas it was “moderate” compared to histology (kappa = 0.41-0.48) and “substantial” compared to culture (kappa = 0.61-0.8). The iRUT was slightly less sensitive than the cRUT, however, the iRUT was easy to prepare, had a shelf-life of up to 5 months and the unit cost was approximately 20 times cheaper than the cRUT. The results indicate that the iRUT may replace the cRUT in our Endoscopy Unit for routine and rapid diagnosis of *H. pylori* infection.

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REFERENCES

Adesanya AA, Oluwatowoju IO, Oyedeji KS, *et al.* Evaluation of a locally-made urease test for detecting *Helicobacter pylori* infection. *Niger Postgrad Med J* 2002; 9: 43-7.

Ansorg R, Von Recklinghausen G, Pomarius R, *et al.* Evaluation of techniques for isolation, subcultivation, and preservation of *Helicobacter pylori*. *J Clin Microbiol* 1991; 29: 51-3.

Cifuentes P, Topor J, Avagnina A, *et al.* [Validation of a rapid urease test for the diagnosis of *Helicobacter pylori* infection]. *Acta Gastroenterol Latinoam* 2002; 32: 29-34.

Cohen H, Laine L. Endoscopic methods for the diagnosis of *Helicobacter pylori*. *Aliment Pharmacol Ther* 1997; 11 (suppl 1): 3-9.

Das SS, Bain LA, Karim QN, *et al.* Rapid diagnosis of Campylobacter pyloridis infection. *J Clin Pathol* 1987; 40: 701-2.

Fabre R, Sobhani I, Laurent-Puig P, *et al.* Polymerase chain reaction assay for the detection of *Helicobacter pylori* in gastric biopsy specimens: comparison with culture, rapid urease test, and histopathological tests. *Gut* 1994; 35: 905-8.

Forman D, Webb P, Parsonnet J. *H. pylori* and gastric cancer. *Lancet* 1994; 343: 243-4.

Goldie J, Veldhuyzen van Zanten SJ, Jalali S, *et al.* Optimization of a medium for the rapid urease test for detection of *Campylobacter pylori* in gastric antral biopsies. *J Clin Microbiol* 1989; 27: 2080-2.

Hazell SL, Borody TJ, Gal A, *et al. Campylobacter pyloridis* gastritis I: Detection of urease as a marker of bacterial colonization and gastritis. *Am J Gastroenterol* 1987; 82: 292-6.

Hazell SL, Markesich DC, Evans DJ, *et al.* Influence of media supplements on growth and survival of *Campylobacter pylori*. *Eur J Clin Microbiol Infect Dis* 1989; 8: 597-602.

Heatley RV. The *Helicobacter pylori* handbook. Leeds: Blackwell Science, 1995.

Ho AS, Young TH, Shyu RY, *et al.* The accuracy of the rapid urease test and 13C-urea breath test in the diagnosis of *Helicobacter pylori* infection. *Zhonghua Yi Xue Za Zhi (Taipei)* 1996; 58: 400-6.

Kawanishi M, Fukuda S, Kawaguchi H, *et al.* Significance of rapid urease test for identification of *Helicobacter pylori* in comparison with histological and culture studies. *J Gastroenterol* 1995; 30: 16-20.

Kisa O, Albay A, Mas MR, *et al.* The evaluation of diagnostic methods for the detection of *Helicobacter pylori* in gastric biopsy specimens. *Diagn Microbiol Infect Dis* 2002; 43: 251-5.

Kuo CH, Wu DC, Lu CY, *et al.* The media of rapid urease test influence the diagnosis of *Helicobacter pylori*. *Hepatogastroenterology* 2002; 49: 1191-4.

Laine L, Estrada R, Lewin DN, *et al.* The influence of warming on rapid urease test results: a prospective evaluation. *Gastrointest Endosc* 1996; 44: 429-32.

Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33: 159-74.

Li YH, Guo H, Zhang PB, *et al.* Clinical value of *Helicobacter pylori* stool antigen test, Immuno Card STAT HpSA, for detecting *H. pylori* infection. *World J Gastroenterol* 2004; 10: 913-4.

Liao CC, Lee CL, Lai YC, *et al.* Accuracy of three diagnostic tests used alone and in combination for detecting Helicobacter pylori infection in patients with bleeding gastric ulcers. *Chin Med J* 2003; 116: 1821-6.

Lim LL, Ho KY, Ho B, *et al.* Effect of biopsies on sensitivity and specificity of ultra-rapid urease test for detection of *Helicobacter pylori* infection: a prospective evaluation. *World J Gastroenterol* 2004; 10: 1907-10.

Logan RP, Walker MM. ABC of the upper gastrointestinal tract: Epidemiology and diagnosis of *Helicobacter pylori* infection. *Br Med J* 2001; 323: 920-2.

McNulty CA, Dent JC, Uff JS, *et al.* Detection of *Campylobacter pylori* by the biopsy urease test: an assessment in 1445 patients. *Gut* 1989; 30: 1058-62.

Mobley HL, Cortesia MJ, Rosenthal LE, *et al.* Characterization of urease from *Campylobacter pylori*. *J Clin Microbiol* 1988; 26: 831-6.

Montes H, Salmen S, Dolfo W, *et al.* Evaluation of a liquid urease test (LUT) for detection of *Helicobacter pylori*. *Acta Gastroenterol Latinoam* 2003; 33: 73-6.

Morio O, Rioux-Leclercq N, Pagenault M, *et al.* Prospective evaluation of a new rapid urease test

(Pronto Dry) for the diagnosis of *Helicobacter pylori* infection. *Gastroenterol Clin Biol* 2004; 28: 569-73.

National Committee for Clinical Laboratory Standards., Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, approved standard. 2nd ed. M31-A2. Wayne(PA): The committee, 2002.

Onders RP. Detection methods of *Helicobacter pylori:* accuracy and costs. *Am Surg* 1997; 63: 665-8.

Pajares-Garcia JM. Diagnosis of *Helicobacter pylori*: invasive methods. *Ital J Gastroenterol Hepatol* 1998; 30 (suppl 3): S320-3.

Parsonnet J. Gastric adenocarcinoma and *Helicobacter pylori* infection. *West J Med* 1994; 161: 60.

Said RM, Cheah PL, Chin SC*, et al.* Evaluation of a new biopsy urease test: Pronto Dry, for the diagnosis of *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 2004; 16: 195-9.

Thillainayagam AV, Arvind AS, Cook RS*, et al.* Diagnostic efficiency of an ultrarapid endoscopy room test for *Helicobacter pylori*. *Gut* 1991; 32: 467-9.

Vaira D, Holton J, Cairns S*, et al.* Urease tests for *Campylobacter pylori*: care in interpretation. *J Clin Pathol* 1988; 41: 812-3.

Viiala CH, Windsor HM, Forbes GM*, et al.* Evaluation of a new formulation CLOtest. *J Gastroenterol Hepatol* 2002; 17: 127-30.

Xia H, Keane CT, Beattie S*, et al.* Standardization of disk diffusion test and its clinical significance for susceptibility testing of metronidazole against *Helicobacter pylori. Antimicrob Agents Chemother* 1994; 38: 2357-61.