ENZYME-LINKED IMMUNOSORBENT ASSAY FOR SERODIAGNOSIS OF *HELICOBACTER PYLORI* IN DYSPEPTIC PATIENTS AND VOLUNTEER BLOOD DONORS

Wutichai Deankanob[1], Chariya Chomvarin[1], Chariya Hahnvajanawong[1], Pewpan M Intapan[2], Suwin Wongwajana[1], Pisaln Mairiang[3], Churairat Kularbkaew[4] and Apichat Sangchan[3]

[1]Department of Microbiology, [2]Department of Parasitology, [3]Gastroenterology Unit, Department of Medicine, [4]Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Abstract. *Helicobacter pylori*, an important etiological agent in the development of gastritis, peptic ulcer and gastric carcinoma, can be detected by the enzyme-linked immunosorbent assay (ELISA). Our objectives were: 1) to evaluate the efficacy of a commercial ELISA kit (Pyloriset EIA-G III) in sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for diagnosis of *H. pylori* infection in Thai dyspeptic patients in Khon Kaen Thailand; and 2) to examine the seroprevalence of *H. pylori* among blood donors at Srinagarind Hospital's Blood Bank, Khon Kaen University, by the commercial ELISA. Gastric biopsies obtained from 137 dyspeptic patients were diagnosed by culture, rapid urease test (RUT) and histology. Serum samples from the same dyspeptic patients and 100 healthy blood donors were assayed using the commercial ELISA. *H. pylori* infection in dyspeptic patients was considered positive when the culture or both RUT and histology were positive. Using a cut-off value at a titer of 20 U/ml (as recommended by the manufacturer), we found the commercial ELISA kit had a sensitivity of 93.3%, specificity of 75.3%, PPV of 74.7%, NPV of 93.5% and accuracy of 83.2%. The overall *H. pylori* seroprevalence in the healthy blood donors was 57%. Of the 100 healthy blood donors, 39 (60.9%) of the males and 18 (50.0%) of the females were seropositive.

INTRODUCTION

H. pylori is an important etiological agent of gastritis, peptic ulcer disease and gastric carcinoma (Ansorg *et al*, 1991; Parsonnet, 1994; Logan *et al*, 2001). There are several techniques available for the detection of *H. pylori*, including the rapid urease test (RUT), culture and histological analysis. However, all these assays require gastric biopsy specimens from endoscopy, which is invasive (Fabre *et al*, 1994; Heatley, 1995; Kisa *et al*, 2002).

Correspondence: Chariya Chomvarin, Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.
Tel: 66-43-363808; Fax: 66-43-348385
E-mail: chariya@kku.ac.th

A serological assay is a non-invasive alternative for the diagnosis, screening and epidemiological study of *H. pylori* infection (Talley and Noack, 1993; van de Wouw *et al*, 1995; Logan and Walker, 2001). Enzyme-linked immunosorbent assay (ELISA) is a non-invasive, simple and cheap method for detecting *H. pylori* infection (van de Wouw *et al*, 1996; Logan and Walker, 2001; Hanvivatvong *et al*, 2002). It has a sensitivity and specificity in predicting *H. pylori* infection in untreated patients as accurate as invasive tests (Talley *et al*, 1991; 1993; van de Wouw *et al*, 1995, 1996; Logan and Walker, 2001).

It has been suggested that the serological test for *H. pylori* be locally validated relative to other methods because assays vali-

dated in one region may not be valid for other regions (Malfertheiner *et al*, 2002; Hoang *et al*, 2004). In Thailand, invasive methods, such as the RUT and histological examination, are more widely used for the diagnosis of this fastidious microorganism than culture and other non-invasive methods (Arnantapunpong, 1999; Phiphitaporn, 1999; Pankongngam, 2001). Although culture is the gold standard for diagnosing *H. pylori* infection, it is time-consuming and has a lower sensitivity than other methods (Hazell *et al*, 1989; Heatley, 1995). Both RUT and histological examination require biopsies, making them invasive. Therefore, a non-invasive method, such as the ELISA test, is a potential alternative for detecting *H. pylori* in Thai dyspeptic patients.

The seroprevalence of *H. pylori* infection in asymptomatic people varies between nations, ages, races, socioeconomic groups, regions and time of study (Andersen *et al*, 1996). The seroprevalence of *H. pylori* infection in the Thai population in some regions has been reported to be higher than in industrialized countries (Perez-Perez *et al*, 1990). Therefore, the epidemiology of *H. pylori* infection in Thailand's regions should be investigated in order to understand the local risk factors for this microorganism.

In this study we compared a commercial ELISA (Pyloriset EIA-GIII, Orion, Diagnostica, Espoo, Finland) which has been shown to have a high sensitivity in other countries (Talley *et al*, 1991; van de Wouw *et al*, 1995; Logan and Walker, 2001) with culture, RUT and histological examination methods in Thai dyspeptic patients in Khon Kaen, Thailand for the diagnosis of *H. pylori* infection. In order to know the epidemiology of *H. pylori* infection in different regions in Thailand, we assessed the seroprevalence of *H. pylori* infection among healthy blood donors at Srinagarind Hospital's Blood Bank, Khon Kaen University, using this same commercial ELISA kit.

MATERIALS AND METHODS

Patients and blood donor subjects

One hundred and thirty-seven 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 March 2003 and January 2005. The subjects were diagnosed as having non-ulcer dyspepsia (NUD), peptic ulcer dyspepsia (PUD), gastric carcinoma (GCA) and other gastrointestinal diseases (GERD, MALT, duodenitis, etc). The group was comprised of 58 males and 79 females with an age range of 18 to 88 years (mean 48.5 years). We excluded patients who had antibiotic therapy, bismuth treatment, proton pump inhibitors, or histamine-blockers within the previous month.

For the blood donor subjects, a total of 100 consecutive donors who were asymptomatic regarding dyspepsia attending Srinagarind Hospital's Blood Bank, Khon Kaen University between March 2003 and January 2005 were randomly included. The group was comprised of 64 males and 36 females with an age range of 17 to 51 years (mean 29.8 years).

The study was approved by the Ethics Committee of Khon Kaen University and performed in accordance with the Declaration of Helsinki. Informed written consent was obtained from all subjects before being included in the study.

Biopsy specimens

Three gastric mucosal biopsy specimens from the antrum and the corpus were obtained from each patient and divided into three parts. Both antral and corpus specimens were used for culture, the rapid urease test (RUT) 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 in 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 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 commercial RUT agar gel. The results were observed and recorded within 24 hours: a positive result 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 sections, 3-4 micron 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.*

Commercial ELISA test (Pylori EIA-G III)

Serum samples taken from those dyspeptic patients and 100 healthy blood donors were assayed for IgG antibodies against *H. pylori.* The tests were performed according to commercial ELISA kit [(Pyloriset EIA-GIII), Orion, Diagnostica, Espoo, Finland]. A positive result was defined as a titer equal to or greater than 20 U/ml, according to the manufacturer's recommendations.

Analysis of test results

Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) for the ELISA test for *H. pylori* were compared with true positive criteria. The chi-square test was used for statistical analysis of *H. pylori* infection in males and females. $P<0.05$ was considered statistically significant.

The criteria for a true positive for *H. pylori* infection were when it showed 1) a positive culture or 2) negative culture but positive RUT and histological examination (Pajares-Garcia, 1998; Liao *et al*, 2003).

RESULTS

Comparison of culture, RUT, histological examination and ELISA for the diagnosis of *H. pylori*

Of the gastric biopsy specimens in 137 dyspeptic patients, *H. pylori* was found by culture, RUT, histological examination, and ELISA in 50 (36.5%), 64 (46.7%), 71 (51.8%), and 75 (54.7%), respectively. Regarding true positive test criteria, *H. pylori* infection was found in 60 (43.8%) of those patients (Fig 1).

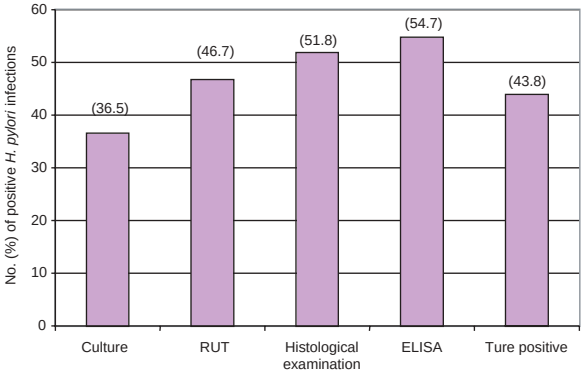

Fig 1–Positive detection rates for *H. pylori* infection in 137 dyspeptic patients detected by culture, RUT, histological examination and ELISA method. The true positive is the culture positive or both the urease and the histological examination positive.

Table 1
Numbers and percentages of *H. pylori* infections detected by the four diagnostic methods.

Culture	RUT	Histological examination	ELISA	Number (%)	Evaluation of infection
+	+	+	+	43 (31.3)	TP
+	+	+	-	3 (2.2)	TP
-	+	+	+	9 (6.6)	TP
+	+	-	+	4 (2.9)	TP
-	+	+	-	1 (0.7)	TP
-	+	-	+	1 (0.7)	FP
-	-	+	+	3 (2.2)	FP
-	+	-	-	3 (2.2)	FP
-	-	+	-	12 (8.8)	FP
-	-	-	+	15 (10.9)	FP
-	-	-	-	43 (31.3)	TN
Total				137 (100.0)	60 (43.8)[a]

TP = True positive, TN = True negative, FP = False positive
TP, culture positive or both urease and histological examination positive
[a]Total numbers (%) of TP

Table 2
Sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), and accuracy of the commercial ELISA test for IgG antibodies to *H. pylori* at different cut-off values among 137 patients.

Cut-off values (U/ml)	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
20[a]	93.3 (56 / 60)	75.3 (58 / 77)	74.7 (56 / 75)	93.5 (58 / 62)	83.2 (114 / 137)
25	91.7 (55 / 60)	76.6 (59 / 77)	75.3 (55 / 73)	92.2 (59 / 64)	83.2 (114 / 137)

[a] Recommended by the manufacturer

Forty-three (31.3%) samples were positive by all four diagnostic methods, 16 (11.7%) by three methods, 5 (3.6%) by two methods, and 30 (21.9%) by only one method (Table 1). The sensitivity, specificity, PPV, NPV, and accuracy of the ELISA test against IgG antibodies at antibody levels higher than 20 U/ml as recommended by the manufacturers and higher than 25 U/ml were compared (Table 2).

Seroprevalence of *H. pylori* infection in blood donors

We determined the seroprevalence of *H. pylori* specific serum IgG among 100 healthy blood donors in Khon Kaen, Thailand. The prevalence of seropositivity among the 100 blood donors was 57%. The seroprevalences of *H. pylori* positivity were 60.9% (36/64) in male and 50% (18/39) in female blood donors.

Table 3
Seroprevalence of 100 healthy blood donors attending the Srinagarind Hospital's Blood Bank, Khon Kaen University, Thailand.

Genders	Number	Age range (year)	Mean age (± SD)	No. of ELISA positive (%)	Titer levels (U/ml)	Median titer levels (U/ml)
Male	64	31-48	30.8 (± 9.1)	39 (60.9)	2 - 262	35
Female	36	17-51	28.0 (± 8.0)	18 (50.0)	3 - 300	23
Total	100	17-51	29.8 (± 8.8)	57 (57.0)	2 - 300	36

Although the infection rate in males was higher than in females, there was no statistically significant difference ($p>0.05$). The median titer level was 36 U/ml (Table 3). The seropositive rates were 54.4% (31/37) and 60.5% (26/43) in the 10-30 and 30-60 year age groups.

DISCUSSION

Alternative serodiagnostic techniques for detection of *H. pylori* infection include bacterial agglutination, passive hemagglutination, complement fixation, indirect immunofluorescence, and ELISA (Lozniewski *et al*, 1996; Malfertheiner, 1999; Monteiro *et al*, 2001; Hoang *et al*, 2004). ELISA is one of the most extensively employed tests, because it is relatively inexpensive, quick, easy to perform, suitable for screening large populations, and capable of detecting class-specific immunoglobulins (Talley *et al*, 1991; van de Wouw *et al*, 1995; Logan and Walker, 2001). ELISA has been widely used in epidemiological studies, including retrospective studies to determine the prevalence or incidence of infection (Logan and Walker, 2001).

In western countries, ELISA kits have been shown to have high sensitivities and specificities (80-95%) (Logan and Walker, 2001), whereas in developing countries, including Thailand, commercial ELISA kits have high sensitivities but low specificities (Perez-Perez *et al*, 1990; Lin *et al*, 1996; Hanvivatvong *et al*, 2002). A higher sensitivity and specificity were reported when using the antigen prepared from local *H. pylori* isolates than the commercial ELISA reagent kits (Perez-Perez *et al*, 1990; Bodhidatta *et al*, 1993).

A previous study in Bangkok evaluated the five commercial ELISAs for detection of antibodies against *H. pylori* in dyspeptic patients, and found high sensitivities (86.3-97.9%) but low specificities (57.9-72.2%). In this study, we found the commercial ELISA test (Pyloriset EIA-G III) had a high sensitivity (93.3%) and an even higher specificity (75.3%) than in a previous report (72%) (Hanvivatvong *et al*, 2002).

The differences of the commercial ELISA tests in sensitivity and specificity may be the result of many factors, including the antigens used, the population group studied, and/or the reference method used. Lack of specificity may result from cross reaction with other organisms and human gastric mucosa (Newell, 1987; Negrini *et al*, 1996). The high sensitivity makes this ELISA kit reliable as a screening test and an alternative to endoscope in dyspeptic patients. There were 19 (13.9%) false positive samples detected by ELISA classified according to positive criteria. These results showed a slightly lower specificity (75.3%) and PPV (74.7%) compared to culture, histology and RUT (data not shown).

When compared to a previous report in Bangkok using a similar commercial ELISA kit based on IgG detection (Pyloriset EIA-G III) to evaluate *H. pylori* infection in serum (Hanvivatvong *et al*, 2002), our result showed a higher specificity (75.3%) than theirs

(57.9%). This may be due to differences in detection time and in the population group investigated.

The seroprevalence of *H. pylori* infection in a population of blood donors in Khon Kaen was high (57%) among the median age group (29.8 years old). Our results agree with a previous report in Thailand that showed the seroprevalence in the Thai community of Nakhon Ratchasima increased to 55% among those 20-30 years of age, and peaked (75%) among the 30-40 year old age group (Perez-Perez *et al*, 1990). In our study, seropositivity in Khon Kaen blood donors age 10-30 was 54.4% and age 30-60 was 60.5%, indicating that with increasing age there is increased risk of acquiring *H. pylori* infection, though our number are lower than a previous report in the 30-40 year old age group (Perez-Perez *et al*, 1990).

The high seroprevalence rate indicates transmission of *H. pylori* commonly occurs in the Thai population. This may influence the accuracy of the ELISA test in diagnosing *H. pylori* infection in Thai dyspeptic patients because of false positives and low specificity. Another caution in using the ELISA test for the diagnosis of *H. pylori* infection is the possibility that the immune response (IgG) against *H. pylori* may persist for a long time (Parsonnet *et al*, 1997). Some previous reports showed the immune response was completely eliminated (100%) 3-12 months after-*H. pylori* eradication (Laheij *et al*, 1998; Koizumi *et al*, 2003). Therefore, ELISA is useful for monitoring the outcome of *H. pylori* eradication therapy. However, it may be difficult to distinguish between past and present infection in the dyspeptic patients who did not eradicate this microorganism.

Regarding the rather high seroprevalence of *H. pylori* in the Khon Kaen population, some previous studies have shown the ELISA test for *H. pylori* detection should be adjusted for the population studied (Bener *et al*, 2002; Hoang *et al*, 2004). Therefore, we tried to use higher cut-off levels (25 U/ml instead of 20 U/ml). However, the specificity and PPV were slightly higher (76.6%) (Table 2), the sensitivity and NPV were slightly lower, but the accuracy remained unchanged. Therefore, increasing cut-off levels in the ELISA test was useful for increasing specificity but this should not be used for screening.

We conclude that this commercial ELISA test can be used as a screening test for the detection of *H. pylori* infection because of its high sensitivity and accuracy. The high seroprevalence of *H. pylori* infection in Khon Kaen blood donors is similar to that found in some rural areas in Thailand, indicating that this microorganism may be commonly transmitted among the Thai population. In future, large-scale population-based studies should determine the source of transmission for *H. pylori.*

ACKNOWLEDGEMENTS

This study was supported by a research grant from the Faculty of Medicine, Khon Kaen University, Thailand. We would like to thank the staff of the Endoscopy Unit and staff at Srinagarind Hospital's Blood Bank (especially Dr Chintana Puapairoj), Khon Kaen University, Thailand, for their help with specimen collection and Mr Bryan Roderick Hamman for his assistance with the English-language presentation of the manuscript.

REFERENCES

Andersen LP, Rosenstock SJ, Bonnevie O, Jorgensen T. Seroprevalence of immunoglobulin G, M, and A antibodies to *Helicobacter pylori* in an unselected Danish population. *Am J Epidemiol* 1996; 143: 1157-64.

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

Arnantapunpong S. *Helicobacter pylori* infection in patients with dyspepsia. *J Rajavithi Hosp* 1999; 10: 17-26.

Bener A, Uduman SA, Ameen A*, et al.* Prevalence of *Helicobacter pylori* infection among low socio-economic workers. *J Commun Dis* 2002; 34: 179-84.

Bodhidatta L, Hoge CW, Churnratanakul S*, et al.* Diagnosis of *Helicobacter pylori* infection in a developing country: comparison of two ELISAs and a seroprevalence study. *J Infect Dis* 1993; 168: 1549-53.

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

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.

Hanvivatvong O, Tatiyakavee K, Thong-Ngam D*, et al.* Detection of antibody in serum and secretion for the diagnosis of *Helicobacter pylori* infection. *J Med Assoc Thai* 2002; 85 : S383-8.

Hazell SL, Markesich DC, Evans DJ, Evans DG, Graham DY. 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.

Hoang TT, Wheeldon TU, Bengtsson C*, et al.* Enzyme-linked immunosorbent assay for *Helicobacter pylori* needs adjustment for the population investigated. *J Clin Microbiol* 2004; 42: 627-30.

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

Koizumi W, Tanabe S, Imaizumi H*, et al.* Effect of anti-*Helicobacter pylori* IgG antibody titer following eradication of *Helicobacter pylori* infection. *Hepatogastroenterology* 2003; 50: 293-6.

Laheij RJ, Witteman EM, Bloembergen P*, et al.* Short-term follow-up by serology of patients given antibiotic treatment for *Helicobacter pylori* infection. *J Clin Microbiol* 1998; 36: 1193-6.

Li YH, Guo H, Zhang PB, Zhao XY, Da SP. Clinical value of *Helicobacter pylori* stool antigen test, ImmunoCard 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 (Engl)* 2003; 116: 1821-6.

Lin TT, Yeh CT, Yang E, Chen PC. Detection of *Helicobacter pylori* by polymerase chain reaction assay using gastric biopsy specimens taken for CLOtest. *J Gastroenterol* 1996; 31: 329-32.

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

Lozniewski A, De Korwin JD, Conroy MC, Plenat F, Weber M. Evaluation of Pyloriset Dry, a new rapid agglutination test for *Helicobacter pylori* antibody detection. *J Clin Microbiol* 1996; 34: 1773-5.

Malfertheiner FM. The year in *Helicobacter pylori* 1999: Current opinion in gastroenterology. Rome: Lippincott Williams & Wilkins, 1999.

Malfertheiner P, Megraud F, O'Morain C*, et al.* Current concepts in the management of *Helicobacter pylori* infection–the Maastricht 2-2000 Consensus Report. *Aliment Pharmacol Ther* 2002; 16: 167-80.

Monteiro L, de Mascarel A, Sarrasqueta AM*, et al.* Diagnosis of *Helicobacter pylori* infection: noninvasive methods compared to invasive methods and evaluation of two new tests. *Am J Gastroenterol* 2001; 96: 353-8.

Negrini R, Savio A, Poiesi C*, et al.* Antigenic mimicry between *Helicobacter pylori* and gastric mucosa in the pathogenesis of body atrophic gastritis. *Gastroenterology* 1996; 111: 655-

65.

Newell DG. Identification of the outer membrane proteins of *Campylobacter pyloridis* and antigenic cross-reactivity between *C. pyloridis* and *C. jejuni. J Gen Microbiol* 1987; 133: 163-70.

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

Pankongngam C. *Helicobacter pylori* and etiology of dyspepsia in Lampang hospital. *Bull Lampang Hosp* 2001; 22: 95-103 (In Thai).

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

Parsonnet J, Replogle M, Yang S, Hiatt R. Seroprevalence of CagA-positive strains among *Helicobacter pylori*-infected, healthy young adults. *J Infect Dis* 1997; 175: 1240-2.

Perez-Perez GI, Taylor DN, Bodhidatta L, *et al.* Seroprevalence of *Helicobacter pylori* infections in Thailand. *J Infect Dis* 1990; 161: 1237-41.

Phiphitaporn S. *Helicobacter pylori*-related peptic disease in Udonthani hospital. *6/2 Regional Med J* 1999; 7: 484-90 (In Thai).

Talley NJ, Newell DG, Ormand JE, *et al.* Serodiagnosis of *Helicobacter pylori*: comparison of enzyme-linked immunosorbent assays. *J Clin Microbiol* 1991; 29: 1635-9.

Talley NJ, Noack KB. The worldwide prevalence of *Helicobacter pylori:* Asmptomatic infection and clinical states associated with infection in adults. In: Goodwin CS, Worsley BW, eds. *Helicobacter pylori* : Biology and clinical practice. Florida: CRC Press, 1993: 63-84.

van de Wouw BA, de Boer WA, Jansz AR, Roymans RT, Staals AP. Comparison of three commercially available enzyme-linked immunosorbent assays and biopsy-dependent diagnosis for detecting *Helicobacter pylori* infection. *J Clin Microbiol* 1996; 34: 94-7.

van de Wouw BA, de Boer WA, Jansz AR, Staals AP, Roymans RT. Serodiagnosis of *Helicobacter pylori* infection: an evaluation of a commercially available ELISA-IgG. *Neth J Med* 1995; 47: 272-7.