Home-Made Rapid Urease Test Compared with Commercial Kit for Diagnosis of *Helicobacter pylori* Infection

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Objective: *To evaluate the sensitivity and specificity of home-made rapid urease test compared with commercial kit for diagnosis of Helicobacter pylori infection.*
Material and Method: *A cross-sectional study of patients who underwent esophagogastro- duodenoscopy from June 2009 to May 2010 was carried out. Gastric biopsy specimens were taken from antrum and body of the stomach for home-made rapid urease testing, commercial kit (hpfast) urease testing, and histological study.*
Results: *One hundred and ninety nine patients were included in the present study. The sensitivity of home-made rapid urease test and hpfast were 40% and 48.5% at 1 hour and 91.4% and 91.4% at 24 hours. The specificity, positive and negative predictive value and accuracy of home-made rapid urease test vs. hpfast were 100% vs. 100%, 100% vs. 100%, 95.5% vs. 95.5% and 96.9% vs. 96.9% respectively. Furthermore 45.3% of home-made rapid urease test and 54.7% of hpfast showed positive results within 1 hour.*
Conclusion: *Home-made rapid urease test has good sensitivity and specificity comparable to commercial kit for the diagnosis of H. pylori infection.*

Keywords: *Helicobacter pylori, Home-made rapid urease test, Hpfast, Diagnostic test*

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Helicobacter pylori is a gram negative bacteria which can live in human stomach in acidic environment and now well established as the causative agent of gastritis, duodenitis, gastric ulcer, duodenal ulcer, gastric mucosa-associated lymphoid tissue (MALT), gastric cancer, and iron deficiency anemia[1,2]. The eradication of this bacteria resolves and prevents the recurrence of these diseases[3,4]. The detection of *H. pylori* infection consists of both invasive and non-invasive methods. The invasive methods require upper endoscopy for gastric tissue including histology, culture, and rapid urease test (RUT), whereas non-invasive methods such as *H. pylori* serology, stool antigen analysis and urea breath test do not require gastric tissue for test[5,6]. Rapid urease tests can detect *H. pylori* infection easily with high sensitivity and specificity. There are many RUT commercial kits available in the market including hpfast (GI supply-USA), CLO test (Kimberly Clark-USA), Pronto Dry (Gastrex-France) and HelicotecUT plus (Strong Biotec Corp-Taiwan). These commercial kits have high sensitivity (90-95%) and specificity (95-100%)[7], but most of them are expensive (6.6-10 USD/test) and may not be available to all clinicians, especially in developing countries. To reduce the cost, many hospitals including HRH Maha Chakri Sirindhorn Medical Center have produced the RUT kit for using in own clinical laboratory.

The purpose of the present study was to evaluate the sensitivity and specificity of home-made RUT produced by HRH Maha Chakri Sirindhorn Medical Center, compared with commercial kit (hpfast) in the diagnosis of *H. pylori* infection.

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Material and Method

Study subjects

A cross-sectional study of patients who underwent esophagogastroduodenoscopy (EGD)

between June 2009 to May 2010 in HRH Maha Chakri Sirindhorn Medical Center, Nakhon Nayok, Thailand were included in the present study. All patients signed their informed consent and received complete physical examination and EGD. Patients were excluded if they had the following criteria: taking proton pump inhibitors, antibiotics or bismuth salts during the previous 4 weeks; prior gastric surgery; severe concomitant diseses and pregnancy or lactation. The present study has already been approved by the institutional ethical committee.

Diagnosis of H. pylori infection

Six gastric mucosal biopsies were taken from both antrum and body of the stomach for RUT and histological examination. Each two specimens (one from antrum and the other one from body) were fully placed immediately in the home-made RUT, hpfast and the other two were sent for histological examination. The home-made RUT (Fig. 1) has cone shape tube which containing 0.5 ml of a freshly prepared test reagent. The reagent was yellowish when the pH was neutral, but it was pink when the reagent was alkaline. The change in color of the media from yellow to deep pink was taken as a positive for home-made RUT. For hpfast (Fig. 2), the reagent was yellowish and green or blue when the reagent was alkaline. The change in color of the media from yellow to green or deep blue was taken as a positive for hpfast. The results of RUT were interpreted at one and twenty four hours at room temperature and the reaction duration was recorded in minutes. Histological examination of gastric tissues was examined by single pathologist with blind to the clinical and laboratory finding of patients. Gastric tissues were fixed in 10% buffered formalin and embedded in parafin for staining with hematoxylin-eosin. Histological examination of gastric body and antral biopsies is the gold standard for the detection of *H. pylori* infection. *H. pylori* positive was defined as the presence of curve rod bacilli, typical feature of *H. pylori* on histological section. A true positive case was defined as a patient who had *H. pylori* detected on histological examination and positive for RUT.

Statistical analysis

Descriptive statistics were used for general data. Quantitative variables were expressed as mean $\pm$ SD and percent for categorical variables. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of home-made RUT and hpfast were calculated accordingly.

Results

One hundred and ninety-nine patients were included in the present study. Demographic and clinical characteristics of the patients are shown in Table 1. The indications for esophagogastroduodenoscopy were dyspepsia 43.2%, upper gastrointestinal bleeding 20.6%, anemia 18.6%, esophageal varice surveillance in cirrhotic patients 8% and others (e.g. follow-up gastric ulcer, gastroesophageal reflux disease with alarm features etc.) 9.6%. The endoscopic findings were normal findings 40.7%, gastritis (with or without duodenitis) 26.6%, gastric ulcer 18.1%, duodenal ulcer 5%, duodenitis 2% and others (e.g. esophageal varices, erosive esophagitis, gastric polyp etc.) 7.5%. The results

Fig. 1 Picture of home-made rapid urease test kit produced by HRH Maha Chakri Sirindhorn Medical Center. The change in color of the media from yellow (right) to deep pink (left) was interpreted as a positive result

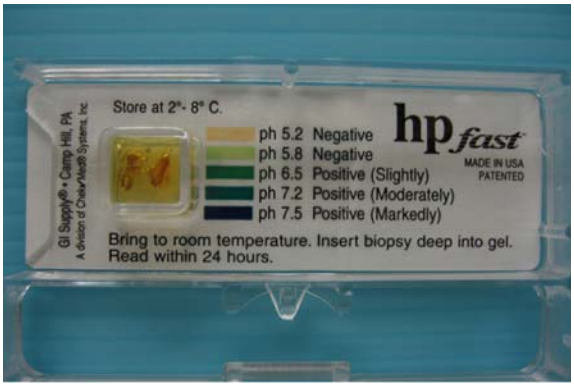

Fig. 2 Picture of commercial rapid urease test (hpfast). The change in color of the media from yellow to green or deep blue was interpreted as a positive result

of home-made RUT and hpfast are shown in Table 2. Further subclassified of RUT results and histological examination according to endoscopic findings are shown in Table 3. Home-made RUT and hpfast have sensitivity at one hour 40% vs. 48.5%, at twenty-four hours 91.4% vs. 91.4%. The specificity, positive predictive value, negative predictive value and accuracy of home-made RUT and hpfast interpreted at twenty-four hours are 100% vs. 100%, 100% vs. 100%, 95.5% vs. 95.5% and 96.9% vs. 96.9% respectively. The mean reaction duration of home-made RUT and hpfast are 180 minutes and 134 minutes respectively. Positive RUT has already changed color within one hour 45.3% for home-made RUT and 54.7% for hpfast.

Discussion

H. pylori is bacteria which can live in human stomach. This bacteria can produce urease for changing urea from dietary protein to ammonia. Ammonia increases environmental pH around bacteria which promote bacteria to live in acidic environment. Both invasive and non-invasive methods are currently available for the detection of *Helicobacter pylori*. The invasive methods require upper endoscopy for gastric tissue including histological examination, culture and rapid urease test. Histological examination of gastric body and antral biopsies remains the gold standard for the detection of *H. pylori* infection[8] and can give the information on morphological features like the presence

Table 1. Demographic and clinical characteristics of patients

Characteristics	Number of patients (n = 199)
Mean age (years)	57.1 ± 16.8
Sex (male: female)	81: 118
Prevalence of *Helicobacter pylori* infection	
Male	37 (46%)
Female	33 (28%)
Indications for esophagogastroduodenoscopy	
Dyspepsia	86 (43.2%)
Upper gastrointestinal bleeding	41 (20.6%)
Anemia	37 (18.6%)
Esophageal varice surveillance	16 (8.0%)
Others	19 (9.6%)
Endoscopic findings	
Normal	81 (40.7%)
Gastritis	53 (26.6%)
Gastric ulcer	36 (18.1%)
Duodenal ulcer	10 (5.0%)
Duodenitis	4 (2.0%)
Others	15 (7.5%)

Table 2. Results of home-made rapid urease test and commercial rapid ureast test (hpfast)

	Home-made RUT	Commercial RUT (hpfast)
Mean reaction duration (minutes) (range)	180 (5-1,320)	134 (1-1,320)
Sensitivity		
At 1 hour	40%	48.5%
At 24 hours	91.4%	91.4%
Specificity		
At 1 hour	100%	100%
At 24 hours	100%	100%
Positive predictive value	100%	100%
Negative predictive value	95.5%	95.5%
Accuracy	96.9%	96.9%

RUT = rapid urease test

Table 3. Subclassified of positive rapid urease test results and histological examination according to endoscopic findings

Endoscopic findings	Home-made RUT	Commercial RUT (hpfast)	Histology
Normal	20 (31.3%)	20 (31.3%)	23 (32.9%)
Gastritis	19 (29.7%)	19 (29.7%)	20 (28.6%)
Duodenitis	1 (1.6%)	1 (1.6%)	1 (1.4%)
Gastric ulcer	13 (20.3%)	13 (20.3%)	15 (21.4%)
Duodenal ulcer	5 (7.8%)	5 (7.8%)	5 (7.1%)
Others	6 (9.4%)	6 (9.4%)	6 (8.6%)
Total	64	64	70

RUT = rapid urease test

or absence of intestinal metaplasia, gastritis, gastric MALT, gastric cancer etc. However, there are many limitations of histological examination such as expensive and time consuming[9]. Culture can give the information about antibiotics sensitivity and resistance of bacteria and guide the clinicians to choose the appropriate treatment regimen, but more demanding in terms of transport, more time consuming and require high experience laboratory which may not be available to general hospital[9]. RUT is inexpensive test, easy to perform and give quick result. The principle of RUT is when place *H. pylori* infected gastric tissue into a urea and pH indicator color reagent containing medium, urease from bacteria will change urea to carbon dioxide (CO_2) and ammonia as equation: urea + $H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$[9]. Ammonia will change color of pH indicator color reagent into specific color depend on type of pH indicator. So the change in color after place gastric tissue into test kit indicate *H. pylori* infection.

In the present study, home-made RUT produced by HRH Maha Chakri Sirindhorn Medical Center has sensitivity 91.4% and specificity 100% comparable with commercial kits available in the market such as CLOtest, hpfast or Pronto Dry which have sensitivity 90-95% and specificity 95-100%[7,8,10,11]. False negative of RUT may occur when low numbers of *H. pylori* are present, patchy distribution of bacteria (biopsy from both body and antral mucosa may increase sensitivity), recent antibiotic or proton pump inhibitors use and upper gastrointestinal bleeding[12,13]. In the present study, we did not exclude patients with upper gastrointestinal bleeding. This may contribute to some of the false negative results. When compare with our previous study[14], positive result within one hour occurs 45.3% in the present study and 62% in the previous study. This maybe explains by the difference in numbers of *H. pylori* between two study population. The advantages of home-made RUT are easy to perform and inexpensive. In HRH Maha Chakri Sirindhorn Medical Center, the cost per one examination of home-made RUT, hpfast, Pronto Dry and histology is 0.3 USD, 8.3 USD, 6.6 USD and 16.7 USD respectively. Furthermore home-made RUT can give a quick result, as 45.3% of the test showed positive result within one hour. This is an important advantage for outpatient where *H. pylori* eradication therapy can be initiated at endoscopic room.

Conclusion

Our home-made RUT has high sensitivity and specificity for the detection of *H. pylori* infection. This RUT is inexpensive and easy to perform. Our home-made RUT should be recommended for routinely use in general practice.

Acknowledgement

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Potential conflicts of interest

None.

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การศึกษาชุดตรวจ rapid urease ที่ผลิตขึ้นเองเปรียบเทียบกับชุดตรวจสำเร็จรูปสำหรับการวินิจฉัยการติดเชื้อ Helicobacter pylori

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วัตถุประสงค์: *เพื่อศึกษาถึงความไวและความจำเพาะของชุดตรวจ rapid urease ที่ทางโรงพยาบาลศูนย์การแพทย์สมเด็จพระเทพรัตนราชสุดาฯ ผลิตขึ้นใช้เองสำหรับการวินิจฉัยการติดเชื้อแบคทีเรีย Helicobacter pylori*

วัสดุและวิธีการ: *เป็นการศึกษาแบบตัดขวางในผู้ป่วยที่มารับการส่องกล้องตรวจทางเดินอาหารส่วนต้น ในช่วงเดือนมิถุนายน พ.ศ. 2552 ถึง พฤษภาคม พ.ศ. 2553 ผู้ป่วยจะได้รับการตัดชิ้นเนื้อของกระเพาะอาหารส่วน body และ antrum ส่งตรวจ rapid urease test โดยใช้ชุดตรวจที่ทางโรงพยาบาลผลิตขึ้นใช้เอง (home-made rapid urease test), ชุดตรวจสำเร็จรูป (hpfast) และส่งตรวจทางพยาธิวิทยา*

ผลการศึกษา: *มีผู้ป่วยเข้าร่วมในการศึกษา 199 คน พบว่าชุดตรวจ rapid urease test ที่ทางโรงพยาบาลผลิตขึ้นใช้เอง และ hpfast มีความไวที่ 1 ชั่วโมง ร้อยละ 40 และ 48.5 ที่ 24 ชั่วโมง ร้อยละ 91.4 และ 91.4, ความจำเพาะร้อยละ 100 และ 100, positive predictive value ร้อยละ 100 และ 100, negative predictive value ร้อยละ 95.5 และ 95.5 และ accuracy ร้อยละ 96.9 และ 96.9 สำหรับการวินิจฉัยการติดเชื้อ H. pylori พบว่าร้อยละ 45.3 ของชุดตรวจ ที่ทางโรงพยาบาลผลิตขึ้นใช้เองและ 54.7 ของชุดตรวจ hpfast สามารถให้การวินิจฉัยได้ภายใน 1 ชั่วโมง*

สรุป: *ชุดตรวจ rapid urease test ที่ทางโรงพยาบาลผลิตขึ้นใช้เองมีความไวและความจำเพาะสูงเทียบเท่ากับชุดตรวจสำเร็จรูปสำหรับการวินิจฉัยการติดเชื้อ H. pylori*