Original Article

THAI J GASTROENTEROL 2005
Vol. 6 No. 2
May - Aug. 2005
55

Comparison of the Accuracy of Two Commercial Rapid Urase Tests, CLOtest® and Pronto Dry®, in Detecting *Helicobacter pylori* Infection

Rojborwonwitaya J, M.D.*
Vijitjunyakul N, M.D.**

ABSTRACT

Background: There were two commercial rapid urease tests available in Thailand, CLOtest® and Pronto Dry®. The comparison between both tests has not been studied widely not only in Thailand but also in all other countries.

Objective: To compare the accuracy of both tests in detecting *Helicobacter pylori* infections.

Patients and Methods: Antral biopsy specimens were done from 200 patients who underwent endoscopic evaluation for dyspeptic symptoms at the Endoscopy Unit, Department of Medicine, Rajavithi Hospital. Six specimens were taken, one for CLOtest®, one for Pronto Dry®, two for culture and two for histological study. The results of both rapid urease tests were determined at 15, 30, 45, 60, 120 minutes and 24 hours intervals. *Helicobacter pylori* infection was defined as 1) positive culture or 2) positive both histology and CLOtest®.

Results: The sensitivity of CLOtest® vs. Pronto Dry® at different intervals were 0.02 vs. 0.35 at 15 minutes; 0.11 vs. 0.47 at 30 minutes; 0.14 vs. 0.55 at 45 minutes; 0.26 vs. 0.65 at 60 minutes; 0.38 vs. 0.71 at 120 minutes and 0.73 vs. 0.87 at 24 hours. The specificity of all tests were 1.0 except for 3 false positive cases in Pronto Dry® group and 1 case in CLO test® groups at 24 hours resulting in the specificity of 0.97 and 0.99, respectively. The accuracy of CLOtest® vs. Pronto Dry® were 0.52 vs. 0.68 at 15 minutes; 0.56 vs. 0.74 at 30 minutes; 0.58 vs. 0.78 at 45 minutes; 0.64 vs. 0.83 at 60 minutes; 0.70 vs. 0.86 at 120 minutes and 0.86 vs. 0.92 at 24 hours. The differences between both methods were statistically significant ($p < .001$).

Conclusion: Pronto Dry® is significantly more accurate than CLOtest® in detecting *Helicobacter pylori* infection at any interval from 15 minutes to 24 hours.

Key words : *Helicobacter pylori*, rapid urease test, accuracy

[Thai J Gastroenterol 2005; 6(2): 55-60]

* *Department of Medicine, Rajavithi Hospital, Bangkok 10400, Thailand*
** *Nonthavej Hospital, Nonthaburi, Thailand*

Background

Helicobacter pylori (H. pylori) was considered to be the most common etiologic agent responsible for peptic ulcer disease both duodenal and gastric ulcers[1, 2] . The detection of *H. pylori* infection requires various modes of laboratory test that produce the variable accuracy which includes both non-invasive and invasive methods[3]. Non-invasive tests such as urea breath test, stool test and serology test are not able to determine the active ulcer disease, and are not well accepted to use for detecting *H. pylori* infection in patient with dyspepsia, especially in patient with alarming symptoms[4]. Invasive tests are the mainstay standard tests in clinical practice, and rapid urea test is the most widely used which is simple and not expensive. CLOtest® is the first commercially available rapid urease test that has been used for more than 10 years in Thailand and still be one of the most popular tests nowadays. Although CLOtest® is commonly used, a few drawbacks are existing. The sensitivity of CLOtest® to detect *H. pylori* infection in Thai patient was only 0.7[5] which is not as high as previous reports from western countries[6]. It also requires up to 24 hours to read the positivity. The recently commercial rapid urease test, Pronto Dry®, is available in our country but its sensitivity and specificity has not been studied in Thai patients. The studies in Malaysia[7] and Korea[8] showed impressive results that it was significantly more accurate than CLOtest®. The objective of our study is to determine the sensitivity, specificity and accuracy of Pronto Dry® comparing to the CLOtest® in Thai patients.

Patients and Methods

The study was performed during February 2001 to January 2002. Two hundred patients, who underwent elective upper gastrointestinal endoscopy for the evaluation of dyspeptic symptoms at Endoscopy Unit, Department of Medicine, Rajavithi Hospital, Bangkok, were included. Patients were excluded if they 1) received proton pump inhibitor, antibiotics, sucralfate or bismuth salt within 4 weeks before the endoscopy; 2) had previous gastric surgery and 3) had previous *H. pylori* eradication.

During the procedure, regardless of the endoscopic finding, six gastric antral biopsy specimens were taken within 2 cm from the pylorus, two specimens for culture, two for histological examination (H & E or immunohistochemistry method if H & E was negative), one for CLOtest® and one for Pronto Dry®. All CLOtest® kits were stored in the refrigerator as recommended by the company but were taken out and left in the room temperature at least 15 minutes before use in order to obtain the best result. Both CLOtest® and Pronto Dry® were read at 15, 30 45, 60, 120 minutes and at 24 hours intervals in the room temperature.

The rapid urease test, both CLOtest® and Pronto Dry®, were considered to be positive if the color changed from amber to pink-red. The gold standard for *H. pylori* infection was defined as positive culture for *H. pylori* or positive both histology and CLOtest® at 24 hour interval.

Statistical Analysis

The sensitivity, specificity and accuracy were used to compare the results of Pronto Dry® and CLOtest® at 15, 30, 45, 60, 120 minutes and 24 hours. The McNemar's test was used to compare the difference.

Results

Two hundred patients, 84 males and 116 females, had the mean age of 50.5 ± 16.27 years. *H. pylori* infection was detected in 99 patients (49.5%). The result of Pronto Dry® in all intervals was shown in the Table 1, and the result of CLOtest® was shown in Table 2.

In Table 3, the sensitivity of Pronto Dry® increased with time from 35/99 tests (35.35%) at 15 minutes to 86/99 tests (86.87%) at 24 hours. No false positive test was detected in all intervals except for 3 tests at 24 hours and it caused the drop of specificity from 100% at the earlier intervals to 97.03% at the 24 hours. The highest accuracy was 92% at 24 hours. The sensitivity at 60 and 120 minutes were significantly lower than at 24 hour but the reading at 24 hours would sacrifice 3 % false positive result.

In Table 4, the sensitivity of CLOtest® varied from 2.02% at 15 minutes to 72.73% at 24 hours and showed the same pattern as Pronto Dry®. The sensitivity at 24 hours was much higher than at 60 or 120 minutes. The specificity is all 100% at 15 to 120 minutes but one false positive test at 24 hours. The highest accuracy was 86% at 24 hours.

The results of both tests showed that the sensitivity and the accuracy of Pronto Dry® were better than

Table 1 Results of Pronto Dry® at different times in comparison with *H. pylori* infection.

Pronto Dry®		*H. pylori* infection (cases)	No *H. pylori* infection (cases)
At 15 min	Positive	35	0
	Negative	64	101
At 30 min	Positive	47	0
	Negative	52	101
At 45 min	Positive	54	0
	Negative	45	101
At 60 min	Positive	64	0
	Negative	35	101
At 120 min	Positive	70	0
	Negative	29	101
At 24 hr	Positive	86	3
	Negative	13	98

Table 2 Results of CLOtest® at different reading times in comparison with *H. pylori* infection.

Results of CLOtest®		*H. pylori* infection (cases)	No *H. pylori* infection (cases)
At 15 min	Positive	2	0
	Negative	97	101
At 30 min	Positive	11	0
	Negative	88	101
At 45 min	Positive	14	0
	Negative	85	101
At 60 min	Positive	26	0
	Negative	73	101
At 120 min	Positive	38	0
	Negative	61	101
At 24 hr	Positive	72	1
	Negative	27	100

of CLOtest® in all time intervals and showed statistical significant difference in all time intervals when determined by the McNemar's student t test. ($p < 0.01$)

Discussion

It is acceptable that *H. pylori* detection is essential in dyspeptic patients with peptic ulcer diseases and with severe erosive gastritis who undergo endoscopy. The *H. pylori* detection in patient with non-ulcer dyspepsia is still controversial because many well-designed studies showed conflicting result about the efficacy of *H. pylori* eradication in symptom improvement in this group of patients[4,9,10]. Although "test and treat" strategy was accepted by some authors[11,12] especially in young patient with no alarming symptoms[13,14], its benefit did not be confirmed[15-17]. Among bundles of controversial information, it is advised to eradicate *H. pylori* in non-ulcer dyspepsia[18-20] especially who had severe symptom[21]. So we recommend detecting *H. pylori* in patients with non ulcer dyspepsia who undergo endoscopy, in order to reduce the cost for additional future investigation for *H. pylori* infection if the treatment failure would occur.

Test for *H. pylori* infection in patient with dyspepsia depends on various invasive and non-invasive

Table 3 Sensitivity, specificity and accuracy of Pronto Dry® at different times.

Time	Sensitivity (%)	Specificity (%)	Accuracy (%)
At 15 min	35.4	100	68
30 min	47.5	100	74
45 min	54.5	100	77.5
60 min	64.7	100	82.5
120 min	70.7	100	85.5
24 hr	86.9	97	92

Table 4 Sensitivity, specificity and accuracy of CLOtest® at different reading times.

Time	Sensitivity (%)	Specificity (%)	Accuracy (%)
At 15 min	2.0	100	51.5
30 min	11.1	100	56
45 min	14.1	100	57.5
60 min	26.3	100	63.5
120 min	38.4	100	69.5
24 hr	72.7	99	86

methods[22-24]. Invasive method by endoscopic examination is still the essential investigation because it can indicate eradication therapy[25]. The rapid urease test, culture and histology are among the most commonly used but each test has high specificity but variable sensitivity. Multiple parallel tests may be done simultaneously to improve the sensitivity[26]. However, the gold standard test is not homogeneous in the literatures, and is variable from many single to many simultaneous tests. Some authors use a positive result from any of these methods: culture, CLOtest® and histology, to verify the infection but others use combinations of any two positives from these three to prevent false positive which may occur[27]. Although, it is very uncommon. Our experience showed that the most common problem of *H. pylori* detection was the low sensitivity. In our study, the gold standard for *H. pylori* infection are positive culture or positive both CLOtest® and histology to avoid false positive and false negative that may occur[28]. We used the culture as one of the gold standard because the biological and biochemical tests of bacteria are specific and false positive from culture method in our institute is rarely possible.

It is more practical and logical to use one single method to detect *H. pylori* infection in daily clinical practice. The rapid urease test is the most widely used to detect *H. pylori* infection and CLOtest® is the most popular commercial test worldwide. The sensitivity of CLOtest®, as reported earlier, was very high and was up to 95% in some reports[28] and was as accurate as the C_{13} urea breath test[29]. However, in our experience its sensitivity in Thai patients was only about 70% as reported previously[5] and only 72.7% in this study. Then the detection of *H. pylori* infection by CLOtest® alone may not be probably acceptable in our country because of its relatively low sensitivity, especially for patients who have complications of peptic ulcer disease, frequent recurrence. CLOtest® alone may not also be used to confirm the eradication as well.

Pronto Dry® is a commercial rapid urease test recently available in Thailand. Its cost is lower than CLOtest® and the recommended reading is at 60 minutes after embedding tissue in the gel, which is sooner than CLOtest® which the recommended reading is at 24 hours. The comparison between Pronto Dry® and CLOtest® had not previously been studied.

According to this study, there was no advantage of Pronto Dry® when reading was done at 60 minutes over CLOtest® when reading was done at 24 hours, their sensitivity were 64.7% vs. 72.7% respectively. However, the sensitivity of Pronto Dry® at 120 minutes was as high as the sensitivity of CLOtest® at 24 hours. Our data also showed that sensitivity of Pronto Dry® was much higher than CLOtest®, both reading at 24 hours, 86.9% vs. 72.7% respectively. Therefore, the recommendation of Pronto Dry® to be read at 60

Table 5 Comparison of sensitivity, specificity and accuracy of culture, Pronto Dry®, CLOtest® and hisltology

Methods	Sensitivity (%)	Specificity (%)	Accuracy (%)
Culture	96.0	100	98
Pronto Dry (24 hrs)	86.9	97	92
CLOtest (24 hrs)	72.7	99	86
Histology	59.6	85.1	77.5

minutes yields the sensitivity that is probably too low to be acceptable, especially when compare to those of CLOtest®. We recommend that Pronto Dry® should be read at 24 hours, the same time interval as CLOtest®, when the sensitivity is very high and much higher than CLOtest® (86.9% vs. 72.7%).

In this study, the sensitivity of culture method in detecting *H. pylori* infection was very high, 95.6% comparing with 59.6% of histology and 72.7% of CLOtest®, which was due to low sensitivity of histology method (59.6%). The sensitivity, specificity and accuracy of each single test were shown in Table 5, the culture showed the highest accuracy followed by Pronto Dry®. The explanation for this might be partly from the gold standard for *H. pylori* infection in this study that favours culture over the others. However the best single test to detect *H. pylori* in our institute is the culture method.

Histological detection of *H. pylori* had lower sensitivity because high false negative rate. However, there are many special staining procedures which have different sensitivity such as H & E, Giemsa's, Warthin Starry, silver or immunohistochemistry. Among many special stains, the immunohistochemistry is the most sensitive method but with higher cost[30]. Many studies concluded that histology is more sensitive than rapid urease test especially with the immunohistochemistry method[30,31] but many *non-pylori Helicobacter* species may be detected by histology as the false positive[32]. Previous study from Thailand indicated the similar sensitivities of histology by Giemsa's stain and CLOtest®[33].

The sensitivity and accuracy of Pronto Dry®, reading at 24 hours, are as high as 86.87 and 92% respectively, and high enough to be a single test for *H. pylori* infection. The optimal time for the highest accuracy of Pronto Dry® should probably be in between 2 hours and 24 hours and further study should be done to obtain the read with maximum accuracy. Therefore, we recommend that Pronto Dry®, more preferable than CLOtest®, to be used as a single test for the detection of *H. pylori* in certain institutes where other tests are not available because its sensitivity is very high and false positive is minimal.

CONCLUSION

In summary, our study indicates that Pronto Dry® is more sensitive and accurate than CLOtest® for detecting *H. pylori* infection at any reading times from 15 minutes to 24 hours with statistical significance. Pronto Dry® is better read at 24 hours when higher sensitivity and accuracy is obtained and can be used as the single test for the detection of *H. pylori* infection.

REFERENCES

1. Tytgat GNJ, Noach LA, Rauws EA. *Helicobacter pylori* infection and duodenal ulcer disease. Gastroenterol Clin North Am 1993; 22: 127-39.
2. Nomura A, Stemmermann GN, Chyou PH, *et al*. *Helicobacter pylori* infection and the risk for duodenal and gastric ulceration. Ann Intern Med 1994; 12: 977-81.
3. Cutler AF, Havstad S, Ma CK *et al*. Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection. Gastroenterology 1995; 109: 136-41.
4. Laine L, Schoenfeld P, Fennetty M. Therapy for *Helicobacter pylori* in patients with nonulcer dyspepsia. A meta-analysis of randomized, controlled trials. Ann Intern Med 2001; 134: 361-9.
5. Rojborwonwitaya J, Patanareungrai A, Chantarakuptankul S, *et al*. The accuracy of the reused CLO® test and CLO® test in detecting *Helicobacter pylori* infection. Poster session in World Congress of Gastroentelogy, Bangkok, Thailand 2002.
6. Desforges JF. *Helicobacter pylori* and peptic ulcer disease. N Engl J Med 1991; 15: 1043-8.
7. Haq G, *et al*. Can diagnosis of *Helicobacter pylori* be rapid and yet sensitive? (Abstract). J Pak Med Assoc 1991; 41: 103-4.
8. Goh KL, et al. The rapid urease test in the diagnosis of *Helicobacter pylori* infection. (Abstract) Singapore Med J 1994; 35: 161-2.

9. Tally NJ, Lauritsen K. The potential role of acid suppression in functional dyspepsia: the BOND, OPERA, PILOT and ENCORE studies. Gut 2002; 50 (Suppl IV): iv36-iv41.
10. Talley NJ, Quan C. Review article: *Helicobacter pylori* and nonulcer dyspepsia. Aliment Pharmarcol Ther 2002; 16 (Suppl 1): 58-65.
11. Meurer LN, Bower DJ. Management of *Helicobacter pylori* infection. Am Fam Physician 2002; 65: 1327-36.
12. Malfertheiner P. *Helicobacter pylori* eradication in functional dyspepsia: new evidence for symptomatic benefit. Eur J Gastroenterol Hepatol 2001; 13 (Suppl 2): S9-S11.
13. 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.
14. Moayyedi P. *Helicobacter pylori* test and treat strategy for young dyspeptic patients: new data. Gut 2002; 50 (Suppl IV): iv47-iv50.
15. Froehlich F, Gonvers JJ, Wietlisbach V, *et al*. *Helicobacter pylori* eradication treatment does not benefit patients with nonulcer dyspepsia. Am J Gastroenterol 2001; 96: 2329-36.
16. Greenberg PD, Cello JP. Lack of effect of treatment for *Helicobacter pylori* on symptoms of nonulcer dyspepsia. Arch Intern Med 1999; 159: 2283-8.
17. Talley NJ, Vakil N, Ballard ED 2nd, *et al*. Absence of benefit of eradicating *Helicobacter pylori* in patients with nonulcer dyspepsia. N Engl J Med 1999; 341: 1106-11.
18. McColl K, Murray LS, El-Omar E, *et al*. Symptomatic benefit from eradicating *Helicobacter pylori* infection in patients with non-ulcer dyspepsia. N Eng J Med 1998; 339: 1869-74.
19. Gilvarry J, Buckley MJM, Beattie S, *et al*. Eradication of *Helicobacter pylori* infection in patients with non-ulcer dyspepsia. Scand J Gastroenterol 1997; 32: 535-40.
20. Verma S, Giaffer MH. *Helicobacter pylori* eradication ameliorates symptoms and improves quality of life in patients on long-term acid suppression. A large prospective study in primary care. Dig Dis Sci 2002; 47: 1567-74.
21. Ching CK, Wong BC. Who should be treated for *Helicobacter pylori* infection? Hong Kong Med J 1999; 5: 151-7.
22. Alan FC, Suzanne H, Chen K, *et al*. Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection. Gastroenterol 1995; 109: 136-41.
23. Andersen LP, Klilerick S, Petersen G, *et al*. An analysis of seven different methods to diagnose *Helicobacter pylori* infection. Scand J Gastroenterol 1998; 33: 24-30.
24. Wong BC, Wong WM, Wang WH, *et al*. An evaluation of invasive and non-invasive tests for the diagnosis of *Helicobacter pylori* infection in Chinese. Aliment Pharmacol Ther 2001; 15: 505-11.
25. Braden B, Caspary WF. Detection of *Helicobacter pylori* infection: when to perform which test? Ann Med 2001; 33:91-7.
26. Saksena S, Dasarathy S, Verma K, *et al*. Evaluation of endoscopy-based diagnostic methods for the detection of *Helicobacter pylori*. Indian J Gastroenterol 2000; 19: 61-3.
27. Vaira D, Gatta L, Ricci C, *et al*. Review article: diagnosis of Helicobacter pylori infection. Aliment Pharmacol Ther 2002; 16 (Suppl 1): 16-23.
28. Viiala CH, Windsor HM, Forbes GM, *et al*. Evaluation of a new formulation CLOtest. Gastroenterol Hepatol 2002; 17: 127-30.
29. 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.
30. Eshun JK, Black DD, Casteel HB, Comparison of immunohistochemistry and silver stain for the diagnosis of pediatric *Helicobacter pylori* infection in urease-negative gastric biopsies. Pediatr Dev Pathol 2001; 4: 82-8.
31. Madani S, Rabah R, Tolia V. Diagnosis of *Helicobacter pylori* infection from antral biopsies in pediatric patients is urease test that reliable? Dig Dis Sci 2000; 45: 1233-7.
32. Rourke JL, Grehan M, Lee A. *Non-pylori helicobacter* species in humans. Gut 2001; 49: 601-6.
33. Suwanagool P, Atisook K, Pongpech P, *et al*. *Helicobacter pylori*: a comparison of CLO test and Giemsa's stain with culture in dyspeptic patients. J Med Assoc Thai 1993; 76: 185-9.