Comparison of the accuracy of two commercial rapid urase tests, CLOtest® and Pronto Dry®, in detecting *Helicobacter pylori* infection.

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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:*** *This study was done in order to compare the accuracy of both tests in detecting the Helicobacter pylori infections.* ***Method:*** *the 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 minute and 24 hour intervals. Helicobacter pylori infection was defined as 1) positive culture or 2) positive both histology and CLOtest®.* ***Result:*** *The sensitivity of CLOtest® vs. Pronto Dry® at different intervals were 0.02 vs. 0.35 at 15 min; 0.11 vs. 0.47 at 30 min; 0.14 vs. 0.55 at 45 min; 0.26 vs. 0.65 at 60 min; 0.38 vs. 0.71 at 120 min and 0.73 vs. 0.87 at 24 hr. The specificity of all tests were 1.0 except for 3 false positive cases in Pronto Dry® group at 24 hours and 1 case in CLOtest® group 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 min; 0.56 vs. 0.74 at 30 min; 0.58 vs. 0.78 at 45 min; 0.64 vs. 0.83 at 60 min; 0.70 vs. 0.86 at 120 min and 0.86 vs. 0.92 at 24 hr. The differences between both methods were statistically significant (p <.001).* ***Conclusion****: 1) Pronto Dry® is significantly more accurate than CLOtest® in detecting Helicobacter pylori infection at any interval from 15 minutes to 24 hours. 2) Pronto Dry® is best evaluated at 24 hour interval when the highest accuracy is obtained.*

Introduction

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 tests that produce the variable accuracy which includes both invasive and non-invasive methods(3). Non-invasive tests such as urea breath test, stool test and serology test are not able to determine the active ulcer diseases, and are not well accepted to use for detecting *H. pylori* infection in patient with dyspeptic symptom, 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. CLO test® 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 test nowadays. Although CLO test® is commonly used, a few drawbacks are existing. The sensitivity of CLO test® 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 reading 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. Our study is to determine the sensitivity, specificity and accuracy of Pronto Dry® comparing to the CLO test® in Thai patients.

Material and Methods

The study was performed at the Endoscopy unit, Department of Medicine, Rajavithi Hospital during February 2001 to December 2001. Two hundred patients who underwent elective upper gastrointestinal endoscopy for the evaluation of dyspeptic symptoms were included in the study. 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 and immunohistochemistry if H&E was negative, one for CLOtest® and one for Pronto Dry®. All the CLOtest® kits were stored in the refrigerator as recommended by the company but all kits 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 min and at 24 hr in the room temperature.

The rapid urease tests, both CLOtest® and Pronto Dry®, were considered to be positive if their color was change from amber to pink-red. The presence of was defined as positive culture for *H. pylori* or positive both histology and CLOtest® at 24 hr interval.

Statistical methods:

The sensitivity, specificity and accuracy were used to compare the results of Pronto Dry® and CLOtest® at 15, 30, 45, 60, 120 min and 24 hr. The McNemar's test was used to compare the difference between these two tests.

Result:

Two hundred patients, 84 males and 116 females, had the mean age of 50.5 $\pm$ 16.27 years. *H. pylori* infection was detected in 99 patients (49.5%). The results of Pronto Dry® and CLOtest® in all intervals comparing with *H. pylori* status were shown in the table 1 and table 2 respectively.

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 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

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

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

Time	Sensitivity (%)	Specificity (%)	Accuracy (%)
At 15 min	35.35	100	68
30 min	47.47	100	74
45 min	54.54	100	77.5
60 min	64.65	100	82.5
120 min	70.71	100	85.5
24 hr	86.87	97.03	92

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

The results of both tests showed that the sensitivity and the accuracy of Pronto Dry® were better than of CLOtest® in all time intervals and showed statistical significant difference in all time intervals when determined by the McNemar's student *t* test. ($P < 0.01$)

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

Time	Sensitivity (%)	Specificity (%)	Accuracy (%)
At 15 min	2.02	100	51.5
30 min	11.11	100	56
45 min	14.14	100	57.5
60 min	26.26	100	63.5
120 min	38.38	100	69.5
24 hr	72.73	99.01	86

Discussion

It is acceptable that detection of *H. pylori* infection is essential in dyspeptic patients with peptic ulcer disease and severe gastritis who undergo endoscopy. The detection in patient who has symptom of non-ulcer dyspepsia is still controversial because many well-designed studies showed conflicting result about the efficacy of anti- *H. pylori* eradication to improve symptoms in this group of patients(4,7,8). Although "test and treat" strategy was accepted by some authors(9,10) especially in young patient with no alarming symptoms(11,12), its benefit did not be confirmed(13,14,15). Among bundles of controversial information, It is advised to eradicate *H. pylori* in non-ulcer dyspepsia(16,17,18) especially who had severe symptom(19). So our recommendation is to detect *H. pylori* in all 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* in patients with dyspepsia depends on various invasive and non-invasive methods(20,21,22). Invasive method via endoscopic examination is still the essential investigation because it can indicate the diagnosis and eradication therapy(23). the rapid "bedside" urease test, culture and histology are among the most commonly used. Each test has high specificity but variable sensitivity(24). Multiple parallel tests may be done simultaneously to improve the sensitivity(25). However the gold standard test is not homogeneous in the literatures, and is variable from many single to many simultaneous tests. Some authors used a positive result from any of these methods but two positives from these three, culture and CLOtest® positive, histology and culture positive or histology and CLOtest® positive, to prevent false positive result which may occur(26) though it is very uncommon. Our experience showed that the most common problem of *H. pylori* detection was the low sensitivity than the low specificity of tests. In our study, the gold standard for *H. pylori* infection is limited to positive culture or positive both CLOtest® and histology to avoid false positive and false negative that may occur(27). We use 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 not 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 method alone to detect *H. pylori* infection and CLOtest® is the most popular bedside commercial test worldwide. The sensitivity of CLOtest®, as reported earlier, was very high and was up to nearly 95% in some reports(27) and was as accurate as the C13 urea breath test(28). However, in our experience its sensitivity in Thai patients was only about 70% as reported previously(5) and only 72.73% in this study. Then the detection of *H. pylori* infection by using CLOtest® alone should not be acceptable in our country because of its low sensitivity, especially for patients who have complications of peptic ulcer disease, frequent recurrence or whose ulcer recurrence from *H. pylori* are associated with high morbidity. CLOtest® alone should not also be used to confirm the eradication.

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 min after sampling which is sooner than CLOtest® which the recommended reading is at 24 hr. 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 min over CLOtest® when reading was done at 24 hr, their sensitivity were 64.65% VS 72.73% respectively. However the sensitivity of Pronto Dry® at 120 min was as high as the sensitivity of CLOtest® at 24 hr. Our data also showed that sensitivity of Pronto Dry® was much higher than CLOtest® at 24 hr, 86.87% VS 72.73% respectively. Therefore, the recommendation of Pronto Dry® to be read at 60 min yields the sensitivity that is too low to be acceptable, especially when compare to those of CLOtest® at 24 hr. We recommend that Pronto Dry® should be read at 24 hr, the same time interval as CLOtest®, when the sensitivity is very high and much higher than CLOtest®.

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 CLOtest®, which was due to very low sensitivity of histology (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®. So the best single test to detect *H. pylori* in our institute is the culture.

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

Methods	Sensitivity (%)	Specificity (%)	Accuracy (%)
Culture	95.96	100	98
Pronto Dry® (24 hrs)	86.87	97.03	92
CLOtest® (24 hrs)	72.73	99.01	86
Histology	85.14	59.59	77.5

Histological detection of *H. pylori* had lower sensitivity because high false positive rate by our gold standard criteria. However there are many special staining procedures which have different sensitivity such as H&E, Giemsa's, Warthin Starry, silver or immunohistochemistry stain. Among these many special stains, the immunohistochemistry is the most sensitive method (29) but with higher cost. Many studies concluded that histology is more sensitive than rapid urease test especially immunohistochemistry stain(29, 30) but many non-pylori Helicobacter species may be detected by histology alone as the false positive(31). Previous study from Thailand indicated the similar sensitivities of histology by Giemsa's stain and CLOtest®(32).

The sensitivity and accuracy of Pronto Dry®, reading at 24 hours are as high as 86.87 and 92% respectively, and are high enough to be a single test for *H. pylori* infection. Therefore we recommend Pronto Dry®, more preferable than CLOtest®, to be used as a single test for the detection of *H. pylori* infection, in certain institutes where the 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 a 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 best read at 24 hours when the highest sensitivity and accuracy is obtained and can be used as the single test for the detection of *H. pylori* infection.

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