Diagnostic properties of expired and reused negative expired ASAN® Helicobacter Test

ORIGINAL ARTICLE

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ABSTRACT

OBJECTIVE

To evaluate the efficacy of expired ASAN® Helicobacter Test (CLO test) and a color-changing property of negative expired CLO test.

METHODS

This cross-sectional diagnostic study was conducted at Kalasin Hospital, Thailand. The negative expired CLO test pellets were obtained from the patients undergoing endoscopy from December 2017 to March 2018. Gastric mucosa from positive expired CLO test pellets was reinserted into the negative expired CLO test pellets. Color changing, the interval after expiration and the interval from the first use were recorded. Pathological examination was used as a gold standard.

RESULTS

We test the diagnostic property of 20 negative reused expired CLO test pellets. It yielded 92.9% sensitivity, 76.5%s specificity, 62.0% positive predictive value, 96% negative predictive value, and 81.3% accuracy. All test pellets had a color changing from yellow to magenta within 1 to 4 hours after reinsertion with the positive CLO test gastric mucosa. The median interval from the first use was 15 days (interquartile range (IQR), 5 to 28), the median expired interval was 139 days (IQR, 9 to 191).

CONCLUSION

This was the first study that tried to confirm the diagnostic property of the expired CLO test. Its color-changing property of negative expired CLO test remained with high sensitivity and high negative predictive value.

INTRODUCTION

Since 1985 by Barry Marshall and Robyn Warren, *Helicobacter pylori* (HP) has been recognized as the causation of dyspepsia.[1] It is the most common gastrointestinal problem in general practice and contributes for 23.7% indication for the esophagogastroduodenoscopy (EGD).[2-4] Campylobacter-like organism (CLO) test is one of the common tests for diagnosis of HP with 80-100% of sensitivity and 97-99% specificity.[5-7] Theoretically, unchanged substrate in a negative expired CLO test can change its color from yellow to magenta after insertion of the new HP infected gastric mucosa which the sensitivity ranging from 80 to 95%, and the accuracy is found to be as high as 98.3%.[8-13] However, the previous studies never mention the accuracy of expired CLO test which occasionally occurs in the settings of a limited resource. Thus, the aim of this study is to test the accuracy of expired CLO test for the diagnosis of HP infection and assess the capability of color change of negative expired CLO test.

METHODS

STUDY DESIGN AND OVERSIGHT

This is a cross-sectional diagnostic test. This study was conducted at Endoscopic Unit, Kalasin Hospital, Northeastern Thailand. The area had the highest prevalence of HP in the country, 60.6%, whereas 46.9% in Northern, 39.0% in Central, 14.4% in Southern Thailand.[14]

PATIENTS

The inclusion criteria were patients 18 years of age or older undergoing endoscopy regardless

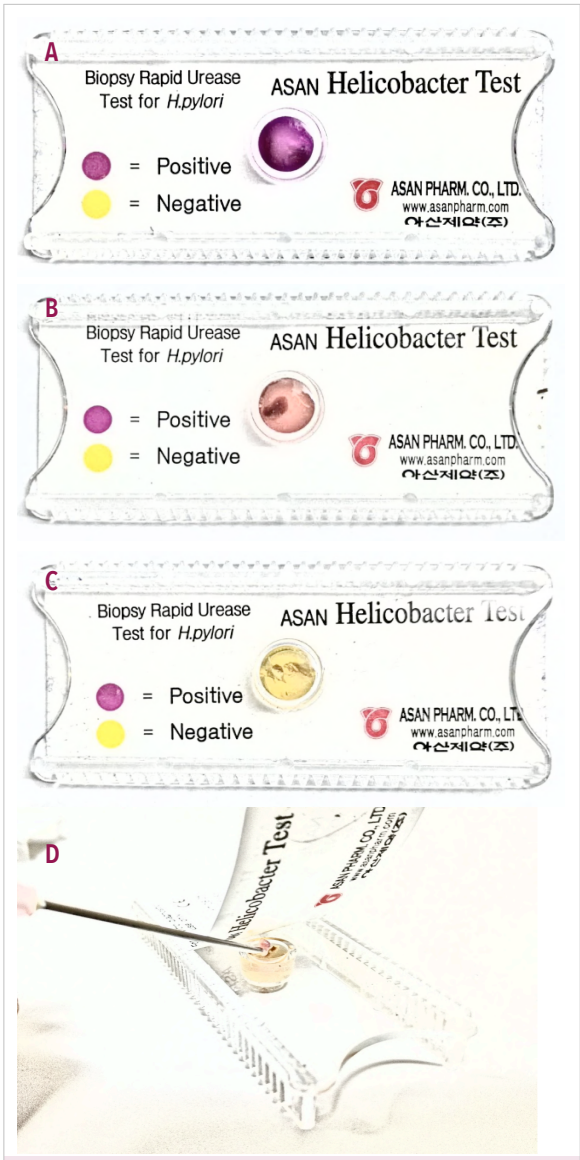


Figure 1. ASAN® Helicobacter Test
Panel A, magenta or positive CLO test; Panel B, equivocal color; and Panel C, yellow or negative CLO test; and Panel D, insertion technique

indications from December 2017 to March 2018, although the common indications were upper gastrointestinal bleeding (34%), chronic dyspepsia (32%) and the screening for esophageal varices (15%). The author biopsied 4 pieces of gastric

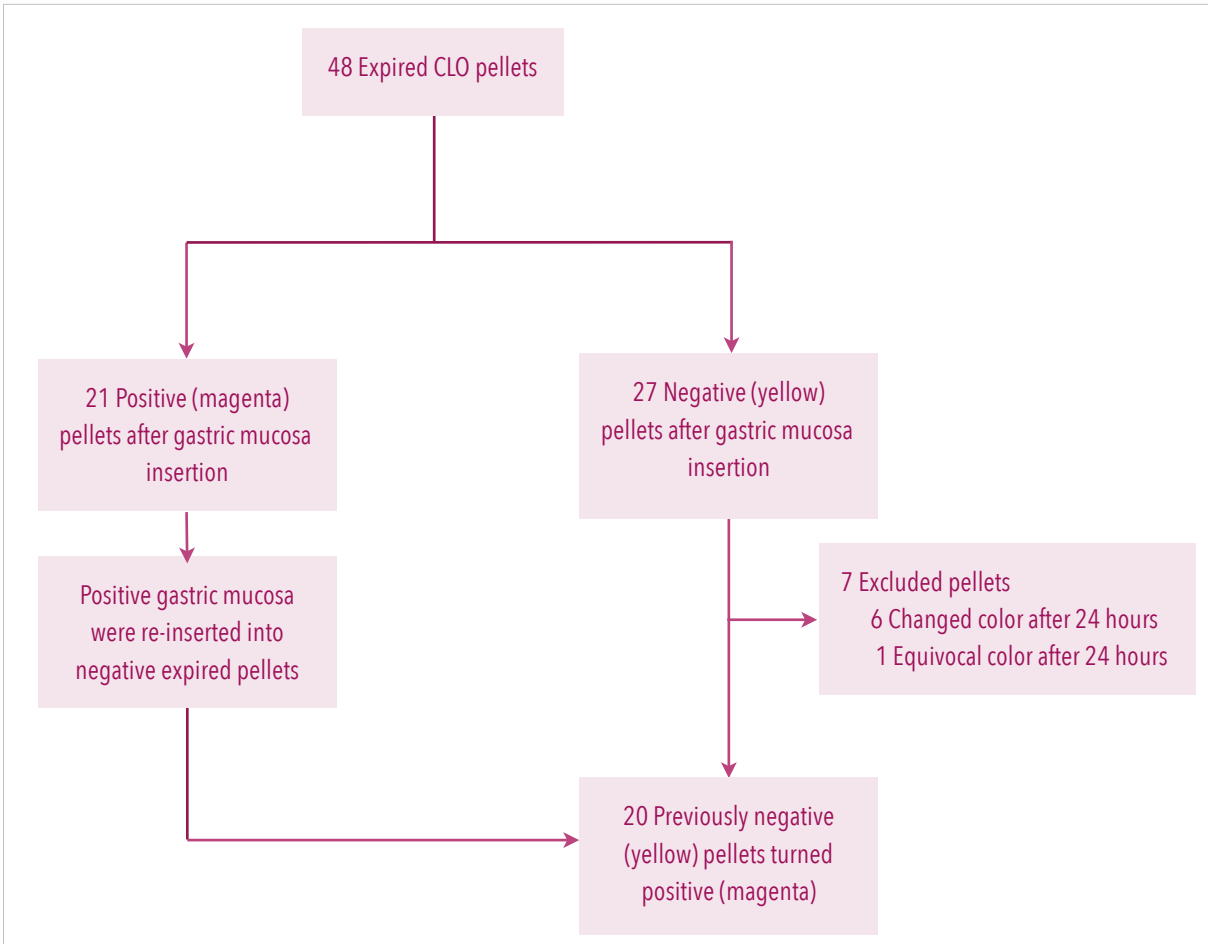


Figure 2. Flow of the study

mucosa without blood contamination, 2 pieces from antrum and 2 pieces from the gastric body, each pair of the antrum and gastric body mucosa was used for pathological examination and CLO test. Whenever expired CLO test pellets turned positive (Figure 1), the author picked up one piece of positive gastric mucosa and inserted into the negative expired CLO test pellets immediately without removing the old gastric mucosa. Color changing, an interval between insertion time and color changing time were recorded. All CLO test pellets were left at room temperature for observation of up to 24 hours.

REUSED CLO TEST® PALLETS

The author collected reused negative expired CLO test pellets (ASAN® pharm. Co., Seoul, Korea) which stored at room temperature at the hospital after the first use (average temperature from December 2017 to March 2018 was 15 to 35 degrees Celsius). The interval from the first use and reuse were recorded. All test pellets were expired, pale or deep yellow (negative test) and they were not damaged or shrinkage. The magenta color pellets (positive test), equivocal color pellets and the pellets that returned magenta after 24 hours were not included in this study (Figure 2).

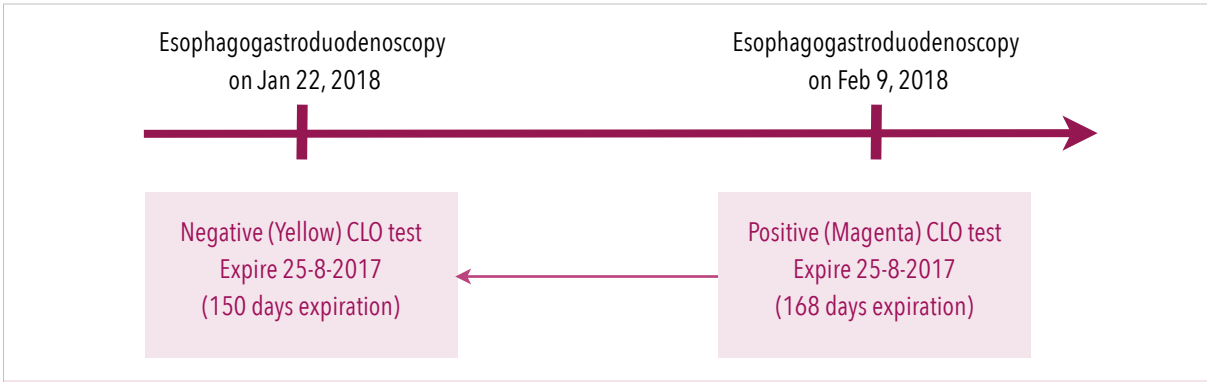


Figure 3. Example of reused CLO test® pallets
In this case, the Esophagogastroduodenoscopy was performed on Jan 22, 2018, after the insertion of gastric mucosa, the test expired reused test was negative. The author still kept this negative pallet. Later on Feb 9, 2018, with the new patient, another reused expired pallet turned positive after gastric mucosa insertion. The positive tissue was, then, re-inserted into the previous negative pallet immediately.

STATISTICAL ANALYSIS

The validity of the reused expired CLO test was interpreted regarding sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) together with accuracy using the pathological examination as a gold standard

RESULTS

Table 1 shows characteristics of the patients contributing their gastric mucosa in the present study. A total of 48 expired pellets were included in this study for the evaluating diagnostic validity of the reused expired CLO test. Primarily, 21 expired CLO test pellets turned positive (magenta) and 27 expired CLO test pellets were still negative (yellow), however, 7 negative expired pellets were excluded due to 6 pellets returned magenta after 24 hours, 1 pellet had equivocal color. Later, the rest 20 negative expired pellets changed color from yellow to magenta within 1 to 4 hours after re-insertion of the positive CLO test gastric mucosa (Figure 3). The median interval from the first use to reuse was 15 days (interquartile range (IQR), 5 to 28 days), the median interval from expiration to reuse was 139 days (IQR, 9 to 91 days). HP infection was confirmed by pathological examination, only 1 from 27 negative expired CLO test was reported HP infection by the pathologist (false negative). All 48 expired CLO test pellets had 92.9% sensitivity (95% confidence interval (CI), 66 to 99), 76.5% specificity (95% CI, 59 to 89), 62% PPV (95% CI, 47 to 75), 96% NPV (95% CI, 79 to 0.99), and 81.3% accuracy (95% CI, 67 to 91) (Table 2).

DISCUSSION

An unchanged substrate in negative expired CLO test pellets can change its color from yellow to magenta as confirmed by previous studies.[8-13] However, expired pellets may be a loss the ability

Table 1. Characteristics of the patients

Characteristic	
Age-yr	55±13
Male sex (%)	46
Indication (%)	
Dyspepsia	32
Gastrointestinal bleeding	28
Anemia with occult blood	6
Screening for esophageal varices	15
Others	19

Plus minus values are means plus minus SD

to change color due to the expiration of substrate i.e., urea, phenol red, buffers, and bacteriostatic agent. To the best of our knowledge, this is the first study to confirm the efficacy of expired CLO test. As a result, expired CLO test has very high sensitivity and NPV. Moreover, the ability to change the color of negative expired CLO test pellets were confirmed. The possibility of color changing in negative expired CLO test pellets could be caused by either urease in HP infected gastric mucosa or ammonia that already presented in the substrate. The maximum reused interval in this study was 28 days.

Table 2. Sensitivity and specificity of expired CLO test

Test result	Pathological confirmation of *Helicobacter pylori*	No pathological confirmation of *Helicobacter pylori*
Positive	13	8
Negative	1	26

In the region that has a higher prevalence of HP infection, the CLO test usage can cause substantial expense to the hospital with limited resources. For instance, at Kalasin Hospital, CLO test usage estimates to be around 40 to 50 pellets monthly, thus reused CLO test can save up to nearly 70,000 THB annually (116 THB per pellet). Both expired and negative expired CLO test pellets have greater benefit for environment and economy. The negative expired CLO test can be safely reused with good infectious control such as package sealing for prevention of contamination, however, some negative expired CLO test kept in room temperature had an unpleasant odor when reopening. The limitations of this study were a small sample size which causes a wide 95% confidence interval. Comparison between expired and unexpired CLO test pellets should be confirmed with the future larger diagnostic study.

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