REUSE OF CLO TEST IN THE DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION

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Abstract: Negative CLO test pellets can be reused repeatedly in diagnostic endoscopy within a short period of time. However, the duration that these pellets can be stored at room temperature before second use remains unclear. A total of 360 patients, 190 males and 170 females, who required a CLO test during endoscopy, were enrolled in this study. Two biopsies were taken from the gastric antrum of each patient, one for testing with a new pellet and the other for testing with a reused pellet. The reused pellets were used randomly and were divided into five groups according to the time interval between their initial and second usages (1, 2, 3, 6, and > 6 mo). When a positive result was found, the time to color change was recorded. Good correlation was noted for nearly all the paired CLO tests in all groups with either both positive or both negative. Only four pairs produced discrepant results. There was no significant difference when the results of both new and reused CLO tests were compared using McNemar's test ($p > 0.05$). In positive pairs, there was no significant difference in the color change time of both tests in all five groups by two-tailed *t*-test ($p > 0.05$); Pearson's correlation and linear regression showed a strong correlation between the color time change in the five groups ($p < 0.0001$). Only 54 of the 427 negative pellets stored for more than 6 months could be reused because most were dried out or no longer yellow in color. In conclusion, negative CLO test pellets may be reused within 6 months after initial usage provided they are stored at room temperature.

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Key words:
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Rapid urease test is the most common test in the diagnosis of *Helicobacter pylori* infection during endoscopy. CLO test, a commercial trade name (Campylobacter-Like Organism), is one of the rapid urease tests used in many endoscopic units. The CLO test has an agar gel well in its pellet, which contains phenol red and urea. In the presence of *H. pylori* in biopsied gastric mucosa, the urea is hydrolyzed into ammonia by the urease in *H. pylori*. The pH change in the agar gel produces a change of color indicating the presence of *H. pylori*. If there is no color change, the substrate has not been consumed which means that the pellet can be reused.

Our previous study found that negative CLO test pellets might be reused repeatedly within a short period of time if there are environmental and economic considerations [1]. We found that reused CLO tests possessed a sensitivity of 98.6% and a specificity of 98.2% compared to new CLO tests. However, during that study, the pellets were reused within 2 to 15 days, and the maximum time period before reuse was not investigated. The present study was conducted to determine how long these pellets may be stored at room temperature before reuse.

Materials and Methods

Over 6 months from May 1999 to August 1999, 360 consecutive patients who required a CLO test (Ballard Medical Products, Draper, UT, USA) during endoscopy were enrolled in this study. Patients who had taken anti-secretory agents or antibiotics within the 2 weeks prior to endoscopy were excluded, as were patients whose stomach had been partially

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resected. However, patients who had a second endoscopy for follow-up after eradication of *H. pylori* at least 1 month later and had been taking non-steroidal anti-inflammatory drugs (NSAIDs) were included. The study group consisted of 190 men and 170 women. Their ages ranged from 15 to 87 years, with a mean age of 50 years. Among them, 72 had gastric ulcers, 117 had duodenal ulcers, 19 had gastric ulcer scars, 141 had duodenal ulcer scars, and 11 had gastritis.

During endoscopy, two biopsies were taken from the gastric antrum: one specimen was used with a new CLO test and the other with a previously used negative CLO test. The previously negative pellets were required to be yellow in color before reuse. Every reused pellet was tested only once. A result was considered positive for either a new or reused test if the color changed from yellow to magenta. When a positive result was found, the color change times for both new and reused CLO tests were recorded and compared.

After their first use, negative pellets were stored in a central air conditioned room maintained at a temperature of 20°C to 25°C. Before this study, we had collected hundreds of negative CLO test pellets. Those that were yellow and where the agar gel had not dried out were collected each morning before daily endoscopy. They were divided into five groups according to the interval between initial and second use. Group A pellets had been stored for 1 month, group B for 2 months, group C for 3 months, and group D for 6 months. Group E pellets had been stored for more than 6 months, ranging from 7 to 13 months, with a mean of 9.8 months. We placed all reused pellets within each group in a single box and they were chosen at random.

McNemar's test, two-tailed *t*-test, Pearson's correlation, and linear regression were used to compare data among groups. The *p* value using both statistical methods was considered significant if it was less than 0.05.

Results

Among the five groups, there were good correlations for nearly all the paired CLO tests. Most pairs were either both positive or both negative (Table). Only four pairs produced discrepant results. In group A, one pair had a positive result only for the new test. In group B, one pair had a positive new test only and the other pair had a positive reused test only. The fourth mismatched pair was in group C, where a reused test was positive. A total of 95 cases in all five groups were followed up after *H. pylori* eradication; 93 eradicated cases were negative for both paired tests and two non-eradicated cases were positive for both paired tests. Ten patients with gastric ulcers and one patient with a duodenal ulcer were taking NSAIDs before their endoscopies and all of their paired tests were negative. There was no significant difference between the results of both new and reused CLO tests by McNemar's test ($p > 0.05$).

In the cases of both positive pairs, the color change time with each CLO test was almost the same in all five groups, as reflected in the results of two-tailed *t*-test ($p > 0.05$) or by Pearson's correlation and linear regression ($p < 0.0001$) (Figure).

Reused pellets stored for less than 6 months were always reusable without dryness of the well as long as they were yellow in color. However, among 427 negative reused pellets that had been stored for more than 6 months, only 54 pellets could be reused, as the others either had a dry pellet well or the color was not yellow enough.

Discussion

H. pylori infection occurs worldwide and can cause considerable illness to humans. Its prevalence is very high in underdeveloped countries [2]. The CLO test, a useful diagnostic test for this infection [3–5], may not necessarily be an inexpensive test in these countries [6, 7], suggesting that the reuse of negative CLO test pellets could reduce costs. Even in developed countries, the recycling of medical waste that is still medically useful and not harmful to the patient is considered environmentally responsible.

We previously confirmed that negative CLO test pellets kept at room temperature could be used repeatedly for 2 to 15 days until consumed [1]. Another study from Puerto Rico also supports this concept [8]. The authors indicated that 50 negative CLO tests might be performed with the same pellet with 100% accuracy for a period of 1 week to 5 months.

Table. Results of all paired CLO tests

Group	Cases (No.)	Interval (mo)	Both negative (No.)	Both positive (No.)	Mean color change time (min)		Mismatch (No.)
					New	Reused	
A	69	1	27	41	243.3	256.3	1
B	80	2	36	42	240.4	233.6	2
C	94	3	47	46	212.2	226.6	1
D	63	6	36	27	292.9	309.3	0
E	54	> 6	19	35	235.3	248.4	0

Interval = period between initial and second usage of a CLO test.

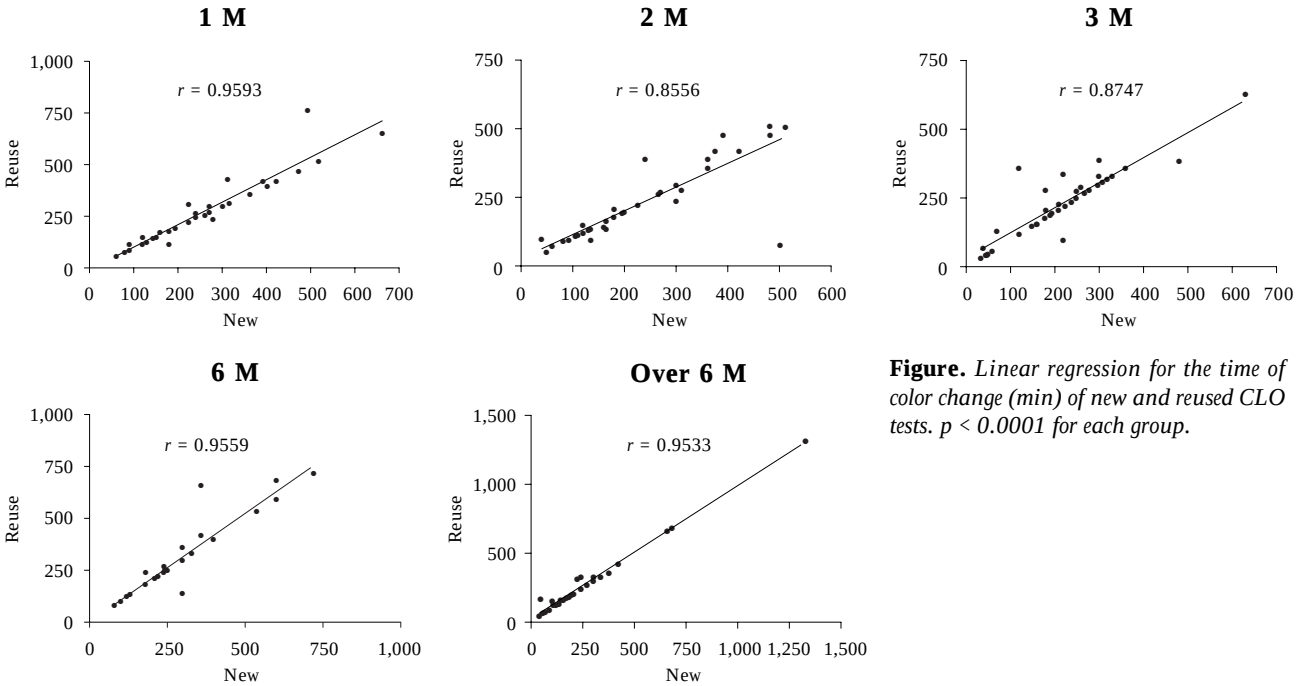

Figure. *Linear regression for the time of color change (min) of new and reused CLO tests. $p < 0.0001$ for each group.*

Our present results indicate that negative pellets can be stored for up to 6 months before second use if they are still yellow in color. Only 54 of the 427 used pellets stored for more than 6 months could be reused; most were dried out or no longer yellow. However, when they were reusable, they still possessed high sensitivity and specificity.

Within 6 months, 99% of reused CLO tests showed satisfactory sensitivity and specificity. The time to color change of reused tests was not significantly different to that in new tests. The mean time to color change for each paired group was less than 6 hours, again not significantly different from new tests. This finding indicates that the substrate in the well of pellets reused before 6 months is unlikely to have been consumed.

As we mentioned in our first report, if the specimen is not well embedded into the agar of the pellet, a mismatch of the test pair may result [1]. Based on this observation, we conducted our second study cautiously. This may explain the excellent results of this study.

In this study, we did not store reused pellets in the refrigerator as we did for new ones. After the first use of a new CLO test, the pellet was kept at room temperature for interpretation. As discussed in our first report [1], we did not warm the pellet to quicken the results, and found that we could reuse each negative pellet an average of 1.5 times. There seems to be no advantage to refrigerating pellets that are to be reused a second or even more times. In addition, both our previous and current study demonstrated satisfactory results using this method.

In conclusion, our results indicate that negative CLO test pellets may be safely reused when stored at room temperature for less than 6 months.

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