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HELICOBACTER PYLORI

Evaluation of a new formulation CLOtest

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Abstract

Background and Aims: The CLOtest® and other rapid urease detection kits are widely used in the endoscopic diagnosis of *Helicobacter pylori*. A new formulation CLOtest has been developed with the goal of obtaining a positive result more rapidly. The aims of this study were to validate the sensitivity and specificity of the new test and compare the time taken for a positive result to be visible in both the new and standard CLOtest.

Methods: Patients presenting for endoscopy at three Western Australian hospitals were prospectively enrolled. Gastric mucosal biopsies were obtained for the standard and new CLOtest and for histology. Grading of color change was conducted by staff blinded to the type of CLOtest used and conducted according to a standardized color chart. *Helicobacter pylori* status was defined by the combination of a positive standard CLOtest and histology, against which the new CLOtest was compared. Results were obtained at 1, 3 and 24 h, and at one center, at 10 min intervals for the first hour.

Results: Three hundred and thirty-five patients were enrolled. Eighty-eight *Helicobacter pylori*-positive individuals were identified. At 24 h, the new test correctly identified all 88, with one false-positive result (sensitivity 100%, specificity 99.6%). At 1 h, sensitivity was 93% with a number of early false-positive results reducing specificity to 96%. Compared to the current CLOtest, the new formulation became positive faster at 20 min ($P=0.001$, $n=51$), but was similar at 1 h ($P=0.06$, $n=88$) and equivalent at 3 h.

Conclusions: The new formulation CLOtest is sensitive and specific, with a trend to give early positive results more quickly, although accuracy at 3 and 24 h is the same.

Key words: *Helicobacter pylori*, urease.

INTRODUCTION

The ability of *Helicobacter pylori* to hydrolyze urea forms the basis of the rapid urease detection tests that are commonly used in the endoscopic diagnosis of *H. pylori* infection. The CLOtest (Ballard Medical Products, Draper, UT, USA) is an agar gel preparation used widely around the world.[1] It offers the ease of a self-contained test kit but does require refrigerated storage and has maximum accuracy when interpreted at 24 h. Warming can be used to speed the urease reaction.[2] Dry format reagent strips have been developed to avoid the need for refrigeration and can be read at 1 h, but it does require the addition of extra reagents.[3,4]

A new formulation CLOtest has been developed with the aim of providing positive results faster, without the need for extra reagents. Data from the manufacturer (available upon request) show that the new formulation remains usable for at least 1 month at room temperature (20–25°C) and, according to accelerated aging studies, lasts twice as long as the usual test. This allows a refrigerated shelf-life of 36 months and less concern about day-to-day usage in the endoscopy room. The study was undertaken to evaluate the sensitivity and

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specificity of the new test against a reference point of a combined standard CLOtest and histology. An additional aim was to compare the time taken for a positive result to be visible in both the new and standard CLOtest, to determine if the new product could offer a quicker, but just as accurate result.

METHODS

Gastroenterology units at three hospitals in Western Australia (Sir Charles Gairdner Hospital (SCGH), Royal Perth Hospital (RPH) and Bethesda Hospital) were involved in the trial, the conduct of which was approved by the institutional ethics committees. Between March 1999 and February 2000, consecutive patients attending for upper gastrointestinal endoscopy were enrolled after providing informed consent. Patients were excluded from the study if they had used bismuth-containing compounds or antibiotics in the preceding 28 days, proton pump inhibitors in the last 7 days or had a history of gastric surgery.

CLOtests were provided in packs, each containing one standard (sCLO) and one new (nCLO) test of identical appearance. Each test was labeled with a randomly generated number to allow subsequent identification of standard and new formulations. Clinicians and other staff involved in the trial were blinded to the formulation of each CLOtest. CLOtests were inoculated with the biopsy sample and placed on a warming apparatus that maintained a temperature of 30–35°C in the gel (manufacturer's (Ballard Medical Products) recommendation). This temperature was maintained over an 8 h daytime period with a return to ambient room temperature at night. An examination of color change was undertaken, with a positive reaction being defined as a definite increase in size of pink coloration around the biopsy or a gradual change in whole gel from yellow to pink. A standardized color chart was used to score the color change at the three institutions. Readings of the CLOtests were obtained at 1, 3 and 24 h. At one of the three hospitals (SCGH), CLOtest readings were also obtained at 0, 10 and 20 min. Samples for histology were stained with hematoxylin and eosin, and toluidine blue.

During endoscopy, three mucosal biopsies were taken from the antrum (one for each CLOtest and one for histology). An additional biopsy for histology was taken from the gastric corpus.

The *H. pylori* status of a patient was defined by the concurrence of sCLO and histology. Hence, a positive patient had both positive sCLO and histology and vice versa. Subjects in whom there was discordance of results were excluded from the primary analysis. A secondary analysis was performed to compare both versions of the CLOtest against a gold standard of histology alone.

Statistical analysis

Descriptive statistics were obtained, with a calculation of sensitivity, specificity, and a positive and negative predictive value. The significance of rate of color change with time, of both formulations, was assessed by using a chi-squared analysis.

RESULTS

A total of 345 patients were recruited at the three institutions. In 10 patients, there was discordance between histology and the standard CLOtest so that these patients were excluded. As a result, 335 CLOtest pairs and histology were available for evaluation (182 from SCGH, 80 from RPH, 73 from Bethesda). Characteristics of the excluded patients are shown in Table 1.

A total of 88 *H. pylori*-positive patients were identified. At 24 h, the nCLO identified all 88 patients, with one false-positive test (Table 2). This yielded a sensitivity of 100% and specificity of 99.6%. Positive (PPV) and negative (NPV) predictive values were 98.9 and 100%, respectively.

At 1 h, the nCLO identified 82 out of 88 positive subjects with six false-negative results. In 10 cases, color change at 1 h indicated a positive result, which later became negative at 24 h. This gave a sensitivity of 93% and specificity of 96%, with PPV 89% and NPV 97.5% (Table 3).

Of 88 *H. pylori* positive patients, the detection rate of true positives at 1, 3 and 24 h by nCLO was 82 (93.2%), 85 (96.6%) and 100%, respectively. This compared to 75 (85.2%), 82 (93.2%) and 100% for the

Table 1 Characteristics of excluded patients

No. patients	sCLO	nCLO	Histology
7	Negative	Negative	Positive
3	Positive	Positive	Negative

Table 2 A 24 h performance of the nCLOtest

	HP positive *n*	HP negative *n*	Total *n*
nCLO positive	88	1	89
nCLO negative	0	246	246
Total	88	247	335

HP, *Helicobacter pylori*; nCLO, new formulation CLOtest.

Table 3 One hour performance of the nCLOtest

	HP positive *n*	HP negative *n*	Total *n*
nCLO positive	82	10	92
nCLO negative	6	237	243
Total	88	247	335

HP, *Helicobacter pylori*; nCLO, new formulation CLOtest.

Table 4 Comparison of rate of change to a positive result ($n=88$)

	1 h* *n* (%)	3 h† *n* (%)	24 h *n* (%)
sCLO	75 (85.2)	82 (93.2)	88 (100)
nCLO	82 (93.2)	85 (96.6)	88 (100)

sCLO, standard formulation CLOtest; nCLO, new formulation CLOtest. *$P=0.06$; †$P>0.5$.

Table 5 Twenty-four hour results using histology as the gold standard

	HP positive *n*	HP negative *n*	Total *n*
nCLO positive	88	4	92
nCLO negative	7	246	251
Total	95	250	345

HP, *Helicobacter pylori*; nCLO, new formulation CLOtest.

sCLO (Table 4). This numerical trend did not reach statistical significance at 1 h ($P=0.06$), and was not present at three or 24 h ($P>0.5$).

At the one center, where changes in color within the first hour were assessed, 51 positive patients were included. The nCLO developed a positive color change earlier, with 23 of 51 (45%) positive compared to 19 of 51 of the sCLO at 10 min (37%, $P<0.02$); 74.5 versus 55% at 20 min ($P=0.001$); and 88 versus 78% at 1 h ($P=0.06$).

By using histology alone as a gold standard, and including 10 subjects with initially discordant results, 95 *Helicobacter pylori*-positive patients were identified in the present study. At 24 h, the nCLO had a sensitivity of 93% and a specificity of 98%, compared to the sCLO with a sensitivity of 93% and a specificity of 99% (Table 5).

DISCUSSION

Our results show that when compared to a dual reference of sCLO and histology, the nCLO has an equivalent sensitivity and specificity at 24 h to alternative rapid urease tests including the original CLOtest and PyloriTek tests (Bard Interventional Products, Billerica, MD, USA).[5–7] The sensitivity and specificity obtained at 1 h are also comparable, although test accuracy suffered from a number of early false-positive results that subsequently became true negatives at the later reading. This may be because of an effect of the changed gel formulation, although a variation in observer interpretation of the color changes could also have been a factor. Management decisions could be based on the 1-h reading, although sensitivity and specificity are further improved by a final reading at 24 h.

In assessing the time taken for the CLOtest to become positive, there was a trend for the nCLO to be faster at 1 h, with the two tests becoming comparable after 3 h; at 1 h, 93% of tests that were ultimately positive had become so, as opposed to 85% with sCLO (Table 4). When we evaluated color change within the first hour, nCLO became positive significantly more quickly, such that at 20 min, 74% of the nCLO, and only 55% of sCLO formulations were positive. However, because this advantage had diminished by 1 and 3 h, the overall clinical advantage is likely to be relatively small.

In the present study, a warmer device was used to provide an optimal reaction time. The effect of warming the CLOtest has been investigated by Laine *et al*,[2] who found that incubating the test at 37°C increased the time to a positive reaction, but usually saved less than an hour of time. Specificity was not improved by warming the CLOtest. In the absence of a warming device, temperature recordings performed adjacent to electrical equipment in the endoscopy suite indicated that a temperature of 30–35°C was often present (data not shown).

The current sCLO has a refrigerated shelf life of 18 months. When exposed to an ambient temperature, it is recommended that it should be used within 14 days. Changes to the composition of the gel reagents in nCLO are reported by the manufacturer to increase the shelf life to 36 months at 2–8°C, and 43 days at room temperature.

The exclusion of discordant results from the primary analysis and the potentially subjective nature of grading early CLOtest color change could be sources of bias in the present study. A secondary analysis of results, by using histology as a gold standard, indicates that the accuracy of the nCLO is still equivalent to current tests.

In conclusion, the present study demonstrates that the new formulation CLOtest is as sensitive and specific as current tests, and has the potential to identify more positives within the first hour. However, in view of a number of early false-positive results, the 24-h reading time remains more accurate in determining the presence of *Helicobacter pylori* infection.

Statement of interest

Mr Chairman and Prof. Marshall have a commercial interest in Tri-Med Distributors, Australian distributors of the CLOtest. Tri-Med Distributors supported the present study.

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