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Successful Recovery of *H. pylori* From Rapid Urease Tests (CLO Tests)

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OBJECTIVE: Culture of *Helicobacter pylori (H. pylori)* and the determination of its antibiotic susceptibility is of increasing importance with the rise in numbers of antibiotic-resistant strains. The aim of this study was to determine whether *H. pylori* could be successfully isolated from antral biopsies used in Rapid Urease Tests (CLOtests) in clinical practice.

METHODS: Antral biopsies from patients undergoing endoscopy were inserted into the gel of CLOtests to determine the *H. pylori* status of the patients. If the CLOtest was positive at the end of the endoscopy session, it was kept at ambient temperature until processed. In the laboratory, biopsies were removed from the gel and cultured on selective and nonselective media. In an attempt to enhance the recovery rate of *H. pylori*, a subset of positive CLOtests were kept at 4°C from the time that the color change was noted until the removal of the biopsy.

RESULTS: One hundred and forty-one positive CLOtests were studied at times between 1 h and 6 h postendoscopy. Culture success was 93% in the 1st hour but fell off sharply after 2 h ($p < 0.001$). Isolation was also improved if positive CLOtests were stored at 4°C and plated out within 4 h ($p < 0.001$).

CONCLUSIONS: *H. pylori* can be successfully cultured from biopsies in CLOtests kept at room temperature within 2 h or within 4 h if kept at 4°C. Thus the antral biopsy in the CLOtest can be usefully retrieved when, in the light of the CLOtest result, the physician wishes to obtain both culture and antibiotic sensitivity results. (Am J Gastroenterol 1999; 94:3181–3183. © 1999 by Am. Coll. of Gastroenterology)

INTRODUCTION

The diagnosis of *Helicobacter pylori* (*H. pylori*) infection in patients undergoing endoscopy is commonly made using a rapid urease test (RUT), histopathology, or bacterial culture. The latter two procedures may take up to 7 days to yield a result and thus are not always carried out in routine practice. However, with the rise in the number of patients failing initial antimicrobial therapy and the increased detection of antibiotic-resistant strains of *H. pylori* (1–3), the use of culture as a diagnostic tool is still of importance. Culture and determination of antibiotic susceptibility is an expensive procedure if carried out routinely on every patient undergoing endoscopy. It has been suggested that savings may be possible if only urease-positive biopsies are sent to the laboratory for isolation of *H. pylori* (4). The aim of this study was to determine whether *H. pylori* could be successfully isolated from antral biopsies retrieved from a RUT widely used in normal clinical practice, the CLOtest (5). This would reduce the need to collect extra biopsies, as well as spare the expense of processing biopsies that were almost certainly *H. pylori* negative.

MATERIALS AND METHODS

Patients undergoing elective endoscopy were routinely tested for *H. pylori* using the CLOtest. At endoscopy an antral biopsy was immediately inserted into the gel of a prewarmed CLOtest (5, 6) and then kept at 37°C on a warmer. CLOtests were examined for a characteristic color change from yellow to red at the end of the endoscopy session. If the CLOtest was positive (red), the CLOtest slide was moved from the warmer and kept at ambient temperature until processed in the laboratory. In an attempt to enhance the recovery rate of *H. pylori*, 36 positive CLOtests were kept at 4°C from the time that the red color change was noted until the biopsy was removed from the CLOtest.

In the laboratory the biopsies were retrieved from the gel, homogenized with a sterile scalpel blade, resuspended in 0.2 ml normal saline, and plated onto three agar plates (7, 8). We used Blood Agar and Wilkins-Chalgren Agar with and without Dent selective supplement (Oxoid, Basingstoke, England). This selective supplement has been developed for the isolation of *H. pylori* and contains vancomycin (2.5 mg/L), cefsulodin (1.25 mg/L), trimethroprim (1.25 mg/L), and amphotericin B (1.25 mg/L) (9). A portion of the mixture was smeared onto a glass slide, Gram stained, and examined at 1000× magnification for the presence of typical curved or comma-shaped Gram-negative rods. When consenting patients were endoscoped at the *H. pylori* Research Clinic, duplicate antral biopsies were also collected for culture. These were obtained at the same time as the CLOtest biopsies but sent to the laboratory in 0.1 ml of normal saline and processed as described.

Table 1. Isolation Rate of *Helicobacter pylori* From Positive CLOtests Stored at Ambient Temperature and at 4°C for 1–6 h Postinsertion into CLOtest Gel

	CLOtest Kept at Ambient Temperature		CLOtest Kept at 4°C	
Time (h)	n	*H. pylori* Isolated (%)	n	*H. pylori* Isolated (%)
1	14	13 (93)	2	2 (100)
2	32	27 (84)	3	3 (100)
3	31	11 (35)	18	14 (78)
4	18	4 (22)	10	7 (70)
5	7	0 (0)	0	0 (0)
6	3	0 (0)	0	0 (0)
	105	55 (52)	36	26 (72)

Inoculated plates were incubated at 37°C in an atmosphere of 10% CO_2 and a relative humidity of 95–100% for 3–8 days. Bacterial colonies were identified as *H. pylori* on the basis of colonial morphology, positive urease, oxidase and catalase tests, and a second Gram stain.

Data were analyzed using the χ^2 test comparing culture success at 1–2 h *versus* success at 3–4 h. Results of biopsies kept at ambient temperature and at 4°C were also compared.

RESULTS

One hundred and forty-one positive CLOtests were examined at times ranging from less than 1 h to 6 h after insertion of the biopsy into the CLOtest gel. In 46 of these (33%) a biopsy transported in saline was also available for culture. Over all, *H. pylori* was successfully isolated from 57% of the CLOtests and from 100% of the biopsies in saline. Culture success from CLOtests was time-dependent, as shown in Table 1. The isolation rate was 93% if the biopsy was removed from the CLOtest that had been kept at room temperature and plated out in the laboratory within the first hour. It dropped to 84% in the second hour but fell off sharply when the time delay between endoscopy and processing in the laboratory exceeded 2 h (see Table 1). The difference between the culture rates at 1–2 h and those at 3–4 h was highly significant ($\chi^2 = 30.90$, $p < 0.0001$). The recovery rates of *H. pylori* from 36 positive CLOtests kept at 4°C from the time that the red color change was noted until processing in the laboratory are also shown in Table 1. The isolation rate at both 3 and 4 h were much higher in the CLOtests kept at 4°C (78% and 70%, respectively), compared with those kept at ambient temperature (35% and 22%, respectively). The difference between the culture rates at 3–4 h from CLOtests kept at room temperature and those at 4°C was highly significant ($\chi^2 14.10$, $p < 0.0001$).

All biopsies had spiral organisms visible on Gram stain after culture but the shape of the organisms seen after removal of the biopsy from the CLOtest varied depending on the time that the biopsy had remained in the CLOtest. Organisms seen in antral tissue 1 h postendoscopy were typically curved or comma-shaped Gram-negative rods, but at 2–4 h after endoscopy many of the bacteria appeared to be more spherical than rod-shaped.

Although *H. pylori* was isolated from 81 of the 141 positive CLOtests, the rate of growth of the organisms was slower than that of the corresponding strain isolated from the biopsy transported to the laboratory in 0.1 ml saline. The colonies were smaller and sparser. This was apparent in all the strains isolated more than 1–2 h after endoscopy. Typically, *H. pylori* can be subcultured on the 3rd or 4th day after plating out, but when *H. pylori* was cultured from CLOtests, it could only be positively identified after a further 2–3 days' incubation in 72% of isolates.

DISCUSSION

H. pylori can be successfully cultured from antral biopsies if the biopsy in the CLOtest is removed and plated out in the laboratory within 2 h of endoscopy. As CLOtests contain a bacteriostatic agent (5), the growth of both *H. pylori* and other bacteria on the biopsy is retarded but *H. pylori* can be isolated if the time period between biopsy removal and plating out is kept to a minimum. Once the biopsy is removed from the CLOtest gel any remaining inhibitory compounds are sufficiently diluted to allow growth of the *H. pylori* organisms.

Another chemical that, in high concentrations, suppresses the growth of *H. pylori* and would eventually kill the organism, is urea. Soltesz *et al*. tested the survival rates of both clinical isolates and reference strains of *H. pylori* in various transport media and found that the addition of 2% (w/v) urea to the medium resulted in loss of viability (10). Both the rapid urease tests and Christensen's urea broth (11), on which they are based, have between 2% and 3% urea present in a buffered medium, which would explain the decrease in the culture success rate relative to the length of time the biopsy is exposed to urea in the CLOtest.

The highly alkaline environment of positive urease tests may also inhibit *H. pylori*. Several authors have reported that *H. pylori* strains are unable to survive when the pH level is above 8.0 (12–15). It has been suggested that this toxic effect at high pH in the presence of urea is due to an imbalance in ammonium metabolism resulting in a lack of α-ketoglutarate necessary for the biosynthesis of amino acids (16).

Our study continues and extends the findings of a smaller study in which *H. pylori* was isolated from biopsies in "Jatrox" urease test tubes (a type of RUT used in Europe) and from duplicate biopsies in Stuart's transport medium (4). These authors reported an isolation rate of 88% from a total of 17 positive patients and that all specimens were cultured within 4 h of endoscopy, but they did not document the exact length of time between removal of each biopsy and culturing.

This present study demonstrates that the gastric biopsies in CLOtests can be retrieved and used for bacterial culture. Similarly, a recent paper has demonstrated that the gastric

biopsies in CLOtests can be removed and used to detect the presence of *H. pylori* with polymerase chain reaction (PCR) (17).

As well as initial diagnosis of *H. pylori*, the CLOtest RUT has a role in proof of cure, giving a sensitivity of 95% and a specificity of 100%, according to El-Zimaity *et al.* (18). In this role the vast majority of patients are likely to be *H. pylori* negative, so it is wasteful to try to culture *H. pylori* from every patient. Ideally, patients who have been treated for *H. pylori* will all undergo noninvasive follow-up testing to confirm cure, *i.e.*, with urea breath test or a stool *Helicobacter* antigen test. However, it is quite common for follow-up testing to be omitted, or for patients to be referred for elective endoscopy at the same time *H. pylori* eradication therapy is given. When such patients undergo endoscopy the CLOtest is the quickest and least expensive way for a busy gastroenterologist to confirm success of therapy.

As treatment of *H. pylori* infection becomes more widespread, the development of antibiotic-resistant organisms is certain to result in increased demand for both culture and antibiotic sensitivity profiles of isolates. When a patient is found to be still infected after treatment and the CLOtest result is positive, the antral biopsy can then be retrieved from the CLOtest and used to obtain culture and antibiotic sensitivity results.

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