Methodology and Transport Medium for Collection of *Helicobacter pylori* on a String Test in Remote Locations

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ABSTRACT

Background. *Helicobacter pylori* can be isolated from patients using the string test but contaminating oral and nasopharyngeal microflora need to be suppressed by rapid plating out onto selective culture media. Recently, use of this diagnostic method was enhanced by using a novel transport medium to collect specimens from subjects in a remote Australian clinic over 1300 km from the laboratory.

Methods. Retrieved string tests were transported to the laboratory in chilled polystyrene boxes in 5 ml screw-cap bottles with 3 ml of a brain heart infusion broth plus antibiotics. These were 20 μg/ml vancomycin, 10 μg/ml trimethoprim, 10 μg/ml cefsulodin, and 10 μg/ml amphotericin B. A comparison was made between subjects who gargled with a chlorhexidine mouthwash before swallowing the string test and those who did not.

Results. Forty-five urea breath test-positive subjects were tested and *H. pylori* was isolated from 34 of them. Successful culture was achieved from string tests that were in transit for up to 29 hours and where the maximum temperature in the transport box was 14 °C. The additional use of a mouthwash had a marked effect on the isolation rate. *H. pylori* was cultured from 75% of subjects who gargled but only from 39% who did not.

Conclusions. This methodology and transport medium can broaden the use of the string test to more remote geographic areas where endoscopy is not feasible so that *H. pylori* isolates may still be obtained for diagnostic and epidemiologic studies. The value of this promising methodology of collection and transport should be assessed in a controlled study.

Keywords. *Helicobacter pylori*, culture, transport, string test, diagnosis.

Culture of the human gastric pathogen, *Helicobacter pylori*, is the most specific method of diagnosing infection [1], and isolation of the organism is also useful for determining antibiotic susceptibilities and for analyzing genotypic differences between isolates. This fastidious bacterium is usually successfully isolated from gastric biopsies within 2–3 hours after endoscopy when transported at room temperature to the nearest laboratory in a screwtop bottle with 0.1–0.5 ml of 0.9% saline. However, if there is a delay in culturing for more than a few hours, the ability to isolate colonies of *H. pylori* declines. Soltesz et al. found that *H. pylori* isolates were nonculturable if kept for more than 6 hours at holding temperatures above 15 °C but that if stored at 10 °C the organisms survived for 2 days or longer [2]. They also noted that higher rates of successful culture were achieved if the exposure to air during sampling, transport, and handling were minimized because of the low oxygen tolerance of *H. pylori.* For this reason, if there is a delay of more than 6 hours before receipt in a laboratory, the ideal transport medium for gastric biopsies is a semisolid medium such as Stuart's transport medium [2,3] or one of the commercially available media such as Portagerm pylori (bioMérieux, Marcy l'Etoile, France) [4,5].

Although the most common method to obtain *H. pylori* for culture remains the use of gastric biopsies collected during endoscopy, gastric mucus samples can be obtained using a swallowed gastric string, which was first reported by Perez-Trallero and colleagues in 1995 [6]. Our laboratory evaluated the use of the Entero-Test Hp (HDC Corporation, CA, USA) in 1999 [7], and since then this test has been repeatedly used in our

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laboratory for over 150 patients. Our routine procedure is that 1 hour after swallowing, the upper 30 cm of the retrieved string is discarded and the remaining string is placed into 3 ml of Brain Heart Infusion Broth (BHIB; Oxoid Ltd, Basingstoke, UK) for transfer to the laboratory. Aliquots of BHIB are plated out onto selective culture media plates usually within 1 hour of retrieval from the patient. As the string is removed from the stomach through the esophagus and mouth, it attracts other contaminating bacteria but these do not interfere with the isolation of *H. pylori*, if the plating out occurs within an hour.

Recently, we adapted this diagnostic method to collect specimens from subjects in a remote rural clinic over 1300 km from our laboratory. Modifications to our routine method were needed to diminish the growth of oral and nasopharyngeal microflora during the transportation to our laboratory, which could result in a delay of up to 24 hours before plating.

Methods

Study Population

The *H. pylori* status of the indigenous population of Australia is largely unknown, so a study was conducted by our laboratory to compare the prevalence of *H. pylori* in urban and rural indigenous West Australians [8]. The rural community selected for testing was at Jigalong, which is on the edge of the Gibson Desert in the East Pilbara region of central Western Australia, 1300 km northeast of Perth. The majority of the population of 300 people were tested for their *H. pylori* status using the urea breath test (UBT).

Once the UBT results were known, some *H. pylori*-positive subjects were approached to have *H. pylori* isolates collected using the string test. The Western Australian Indigenous Health Information and Ethics Committee (WAAHIEC) approved the study and each participant (or their guardian) provided written informed consent at the time of testing. The study was carried out in two periods in April/May and November/December 2003.

Sample Collection

The subjects fasted overnight and the test was performed before breakfast the next morning. As described previously, the subject swallowed the Entero-Test capsule with 100–200 ml of water and the upper end of the string was taped to the subject's cheek for 1 hour [7,9]. After retrieval, the proximal 30 cm of the string, which had been in contact with the oral and nasopharyngeal flora, was discarded. The effect of contamination from these organisms on the remaining 60 cm of string was compared by having one group of the subjects gargled with, but not swallowed, a commercial chlorhexidine gluconate mouthwash (Savacol, Colgate-Palmolive, Sydney, Australia) followed by four mouth rinses with water prior to the test. While the string test was in the stomach, saliva was not swallowed but expectorated into a plastic cup. In the other group there was no protective gargling before swallowing the capsule.

Table 1 Concentration of antimicrobial agents used in transport medium

Antimicrobial agent	Concentration (μg/ml)
Vancomycin	20
Trimethoprim	10
Cefsulodin	10
Amphotericin B	10

Transport Medium and Culture

The 60 cm of string that had been in the subject's stomach was placed in a 5-ml plastic screwtop bottle and covered with 3 ml of a BHIB mixture containing the antimicrobial agents listed in Table 1. This mixture of antibiotics in these proportions is available commercially as Dent selective supplement (Oxoid Ltd, Basingstoke, UK). A vial containing the lyophilized mixture was aseptically diluted with 2 ml sterile distilled water and 24 μl aliquots were added to 3 ml sterile BHIB. This was prepared up to 1 month in advance and kept at 4 °C until needed. The bottles containing the retrieved string tests were sealed in plastic bags and kept chilled during transport to the laboratory in a small 5-l volume polystyrene transport box containing 3 or 4 prefrozen freezer bricks.

On receipt in the laboratory, the temperature within the box was recorded as well as the time delay from string removal to plating out. An aliquot from each BHIB mixture was plated onto two different selective media plates—Wilkins–Chalgren agar with Dent supplement (Oxoid Ltd, Basingstoke, UK) and the commercially available Pylori plates (bioMérieux, Marcy l'Etoile, France). The broth suspensions were concentrated 15-fold

by centrifugation at 14,000 rpm (20,200 × *g*) and an aliquot of each re-suspended pellet was also plated onto the selective media. Inoculated plates were incubated at 37 °C in an atmosphere of 10% CO_2 and a relative humidity of 95–100% for 7–10 days. Bacterial colonies were identified as *H. pylori* on the basis of colonial morphology, positive urease, catalase and oxidase tests, and a Gram stain.

Results

There were a total of 45 subjects (25 male and 20 female subjects) in the study, ranging from ages 8–92, the average age being 44 years. Prior to the string test, all 45 subjects were determined to be *H. pylori*-positive using the UBT. In 34 of them, *H. pylori* was isolated using the string test; however, nine of subjects repeated the test to achieve this result. Thus, the results reported are from 54 string tests used in 45 subjects.

The transport boxes were sent by road and air and the time taken in transit ranged from 13 to 29 hours; the average time delay between removal of the string and plating being 17 hours. On arrival in the laboratory, the average temperature inside the polystyrene box was 9 °C with a range of temperatures between 3 °C and 14 °C.

The addition of the use of a chlorhexidine mouthwash had a marked effect on the successful culture rate of *H. pylori* [Fig. 1]. Initially, 18 subjects swallowed the string test without gargling and *H. pylori* was successfully cultured from seven of them [39%]. After the mouthwash step was added to the methodology, 36 subjects were tested (including nine subjects who had previously carried out the test without gargling) and *H. pylori* was successfully cultured from 27 of them [75%].

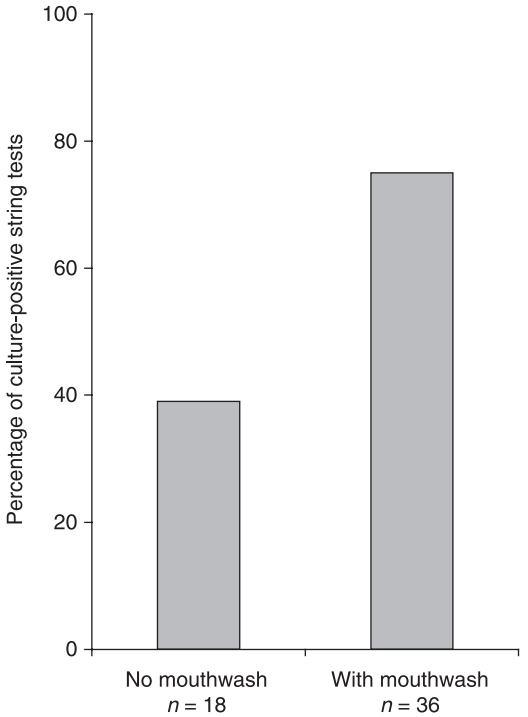

Figure 1 Effect of chlorhexidine mouthwash on *H. pylori* culture rates from a string test.

Discussion

The modifications made to the string test methodology allowed our laboratory to isolate *H. pylori* strains from a remote Australian aboriginal community that had had very little interaction with the non-indigenous population. This transport medium could be used elsewhere in the world to study *H. pylori* strains from other remote areas.

The changes adopted were based on the results reported by previous studies investigating the transportation and survival of *H. pylori* over long time periods [2,4,10]. Most of this work had been carried out either on gastric biopsies or on bacterial isolates of *H. pylori* in vitro. Heep et al. noted that the recovery of *H. pylori* from biopsy specimens was much higher than from bacterial suspensions [4]. This may be because the presence of gastric tissue and mucus has a protective effect or that the bacterial load is much higher in biopsy specimens. The number of *H. pylori* on a string test is undoubtedly also less than in the mucus layer of a biopsy and so the chances of successful culture are lower and the length of time needed for colonies to appear on the agar plates is also longer.

Soltesz et al. noted that higher rates of successful culture were achieved if the exposure to air during sampling, transport, and handling were minimized [2]. For this reason, a small sample bottle (5 ml) with a large volume of broth (3 ml) was used to mimic microaerophilic conditions as closely as possible as there was only a very small volume of air in the bottle after the addition of the 60 cm of moist string.

Initially, in a pilot study in our laboratory (data not reported here), the same concentrations of antibiotics described by Dent and McNulty in 1988 [11] for their *H. pylori*-selective agar medium was tested viz. 10 µg/ml vancomycin, 5 µg/ml trimethoprim, 5 µg/ml cefsulodin, and 5 µg/ml amphotericin B. However, fewer contaminants and more *H. pylori* grew if the original concentrations

were doubled as listed in Table 1. Stevenson et al. tested various combinations of antibiotics to create a new selective medium that could be used to isolate *H. pylori* from animal and food sources where large numbers of competing microorganisms were a problem [12]. These authors showed that the same concentrations of vancomycin, trimethoprim, cefsulodin, and amphotericin B used in our selective transport broth prevented growth of contaminants but still allowed the growth of *H. pylori* on their selective plates. A previous study has reported the use of BHIB with antibiotics as a transport medium to prolong the survival of *H. pylori* in gastric biopsies [10]. This was an enriched broth mixture with 5% horse serum, 0.25% yeast extract as well as 6 μg/ml vancomycin, 4 μg/ml amphotericin B, and 20 μg/ml nalidixic acid.

Prior to this study of indigenous Australians living in a remote community, subjects we have tested had never been asked to use a mouthwash before swallowing the string capsule. This was because all previous subjects were urban residents living in a Western society with plenty of water and knowledge of dental hygiene. These latter two factors as well as the delay in plating out seem to have contributed to the marked difference between *H. pylori* culture rates in subjects who did, and did not, gargle with chlorhexidine mouthwash, i.e., 27/36 [75%] versus 7/18 [39%] as shown in Fig. 1. The use of a mouthwash prior to swallowing the string test may also enhance the success of culture even when there is no requirement for a selective transport medium and no delay in plating out after the removal of the string.

Failure to culture *H. pylori* from certain subjects using this transport medium does not appear to be as a result of the time delay, as all six of the tests that on one occasion took 29 hours to reach the laboratory had *H. pylori* successfully isolated. Similarly the temperature inside this specific transport box was the highest recorded (14 °C) and this did not appear to be a limiting factor. The maximum daily temperatures during the time of our study in Newman, the nearest weather station to Jigalong, ranged from 31.2 °C to 41.4 °C, which are some of the warmest ambient temperatures to which a bacterial transport medium would be subjected anywhere in the world. Previous studies have reported the use of dry ice or the need for storage at a constant temperature of 4 °C [3,4,10].

Although in a controlled trial it would have been ideal to test the use of a mouthwash both with and without a selective transport medium, these reported results show the practical outcomes of the modifications to our methodology made in a remote rural clinic thousands of kilometers from the laboratory. This methodology and transport medium can broaden the use of the string test to more remote areas of the globe where utilization of the endoscope is not feasible, so that *H. pylori* isolates may still be obtained for diagnostic and molecular epidemiologic studies.

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