Effect of transport medium and transportation time on culture of *Helicobacter pylori* from gastric biopsy specimens

R A Veenendaal, A T Lichtendahl-Bernards, A S Peña, H Ph Endtz, C P A van Boven, C B H W Lamers

Abstract

To determine whether transportation time and use of a low budget transport medium (NaCl 0·9 %) would influence culture of *Helicobacter pylori* from gastric biopsy specimens, upper gastrointestinal endoscopy was performed on 42 patients. The specimens were cultured and examined histologically, and *H pylori* antibodies were determined using an ELISA technique. Patients were regarded as *H pylori* positive when culture was positive or when histology or IgG anti-*H pylori* antibodies indicated *H pylori* infection. Rapid transportation of gastric biopsy specimens in NaCl 0·9%, at room temperature resulted in a high diagnostic yield (23 *H pylori* positive cultures in 26 patients with *H pylori* infection). A 24 hour delay in plating gastric biopsy specimens after transportation in NaCl 0·9%, at room temperature, did not seriously affect results (22 instead of 23 *H pylori* positive cultures). The culture results after transportation in Cairy-Blair medium were comparable with those after transportation in NaCl 0·9%, but because of availability, low cost, and ease of handling in the endoscopy department, NaCl 0·9% was preferred as transport medium.

This study shows that for culture of *H pylori* from gastric biopsy specimens sterile saline is an adequate medium, and that transportation can be delayed for 24 hours without a significant loss of diagnostic yield.

(*J Clin Pathol* 1993;46:561–563)

Department of Gastroenterology, University Hospital Leiden
R A Veenendaal
C B H W Lamers

Department of Medical Microbiology, University Hospital Leiden
AT Lichtendahl-Bernards
H Ph Endtz
C P A van Boven

Department of Gastroenterology and Hepatology, Free University Hospital, Amsterdam
A S Peña

Correspondence to:
RA Veenendaal,
Department of Gastroenterology and Hepatology,
University Hospital Leiden,
Building 1, C4-P,
PO Box 9600,
2300 RC Leiden,
The Netherlands

Helicobacter pylori is a curved, spiral Gram negative bacterium that lives in the human stomach. Until now no other reservoir for the bacterium has been identified. Since the successful culture of *Campylobacter pylori*, later renamed *Helicobacter pylori*[1], the bacterium has been established as the major cause of gastritis in humans.[2] At the last world congresses of gastroenterology in Australia a working party reported on the relation between *H pylori* and peptic ulcer disease, proposing a strategy for treatment.[3] A problem in treating *H pylori* is that resistance to several antibiotics has been reported—for example, nitroimidazoles, fluoroquinolones, and macrolides. This makes the need for monitoring of susceptibility to antimicrobials obvious.

Culture of *H pylori*, however, can be difficult because of the fastidious nature of the bacterium and its special characteristics. Rapid transportation, multiple gastric biopsy specimens, and special transport media are considered important for a high diagnostic yield[4,5] making transportation to microbiological laboratories difficult and costly.

Methods

Forty two patients (mean age 51 years, range 22–83, 16 women and 26 men) with severe dyspeptic symptoms were investigated for the presence of gastric *H pylori* infection. Patients who had taken antibiotics, omeprazole, or bismuth-containing drugs during the three months before the study were excluded. Each patient underwent upper gastrointestinal endoscopy at which five biopsy specimens were taken with a sterilised biopsy forceps from the antrum 2 cm proximal to the pylorus in intact mucosa.

One specimen was fixed in 10% buffered formalin, embedded in paraffin wax, and cut into 4 μm thick serial sections, which were stained with haematoxylin and eosin. This specimen was subsequently used for histological identification of *H pylori* and its associated gastritis.

Four adequate gastric biopsy specimens were used for culture. Two gastric biopsy specimens were transported to the microbiology laboratory within two hours in small sterile glass jars, closed with a screw cap, with either 0·2 ml sterile saline 0·9% or Cairy-Blair medium. Two gastric biopsy specimens were transported to the laboratory in the same way but left undisturbed at room temperature for 24 hours. All specimens were processed by direct platting on to the surface of a blood agar plate (Blood Agar Base No 2, Oxoid CM271, containing 5% sheep blood) and inoculation in Skirrow's medium.

Specific IgA and specific IgG antibodies against *H pylori* were measured by a modified enzyme linked immunosorbent assay (ELISA) technique using conjugates labelled with immunoperoxidase specific for human IgA and IgG. For standardisation of the measurement of these antibodies, test conditions were chosen such that the absorbance of the standard reference serum was mean (SD) 0·5 (0·1) for IgA and 1·0 (0·1) for IgG. These values were used to correct the absorbance given by the sera under study. The results were expressed as the absorbance index (AI):

$$AI = \frac{\text{Mean OD reading (n = 2) of patient's serum} - \text{mean OD of blank reading}}{\text{Mean OD reading (n = 2) of reference serum} - \text{mean OD of blank reading}}$$

where OD is the optical density.

An AI of more than 0·32 for anti-*H pylori* IgG and an AI of more than 0·35 for anti-*H pylori* IgA was considered evidence of infection.[6] The antigen preparation, a sonicate of six local prevalent *H pylori* strains, the determination of intra- and interassay variability, and validation of the ELISA technique used have been described elsewhere.[6 7]

Patients were diagnosed as having gastric *H pylori* infection when culture was positive or in the case of a negative culture result, when either histology or serology (IgG anti-*H pylori*) indicated *H pylori* infection.

The χ^2 test for contingency tables was used with Yates' correction.

Results

Forty two patients were studied: 23 had a positive culture result for *H pylori*. In 19 patients culture results of gastric biopsy specimens for *H pylori* were negative. Of these 19 patients, only one had both positive histology and serology (both specific IgA and IgG antibodies) indicating gastric *H pylori* infection; two patients were positive only for *H pylori* on histological examination. The table shows the results of the different diagnostic procedures (transportation times, transport media, serology and histology) for identifying gastric *H pylori* infection. No significant difference (p = 0·87) between the different test methods was found.

When considering the influence of transportation time on gastric biopsy specimens (table), in either sterile saline or Cairy-Blair medium, no significant difference (p = 0·64) was found between rapid and delayed transportation. When considering the influence of the two transport media on culture results of gastric biopsy specimens (table), after rapid transportation or delayed by 24 hours, with storage at room temperature, the difference between these two transport media was not significant (p = 0·87). Four gastric biopsy specimens were taken for bacteriological examination from every patient resulting in positive cultures in 23 patients. In these 23 patients only four had one negative culture of a gastric biopsy specimen while the other three gastric biopsy specimens from the same patient were positive, resulting in 88 positive gastric biopsy culture results out of a possible positive culture total of 92. All four culture negative biopsy specimens (one NaCl, three Cairy-Blair) from *H pylori* culture positive patients were stored for 24 hours at room temperature.

In the other patients all four biopsy specimens showed negative culture results for *H pylori*.

Culture results after different transportation times (<2 h and 24 h) and transport media (NaCl 0·9% and Cairy-Blair) compared with IgA and IgG anti-H pylori antibodies and histological examination of gastric biopsy specimens

	Culture						
	NaCl 0·9%		*Cairy-Blair*		*Serology*		*Histology*
Patients (n = 42)	*<2 h*	*24 h*	*<2 h*	*24 h*	*IgA*	*IgG*	*Haematoxylin and eosin*
H pylori positive	23	22	23	20	19	24	19
H pylori negative	19	20	19	22	23	18	23

χ^2 = 2·45; df = 6; p = 0·87.

Discussion

Although the presence of spiral organisms was first shown in the human stomach in the nineteenth century, it was the successful culture of *H pylori* by Warren and Marshall in 1982 that revived interest and stimulated research all over the world. Culture is probably not the most sensitive test for *H pylori*: it is, however, 100% specific and will in the near future be essential for determining antimicrobial susceptibilities. Despite good in vitro activity[8] many antimicrobial agents, especially in monotherapy, have proved incapable of eradicating *H pylori* from the gastric mucosa partly due to resistance. Testing of *H pylori* for antimicrobial susceptibility is therefore useful in determining and monitoring resistance against metronidazole and nitrofurantoin[9] in individual patients and in the population at large.

To make *H pylori* culture from gastric biopsy specimens possible in every endoscopy unit, efficient and low cost means of transportation to microbiological laboratories are essential. In the past multiple biopsy specimens, rapid transportation, cooling (4°C) and specialised transport media (nutrient broths or buffer solutions) were thought to be essential for good culture results.[4 5 10]

In this study of 92 gastric biopsy specimens from 23 patients with positive gastric cultures for *H pylori*, only four (4·3%) were negative (after 24 hours of transportation). This can be attributed to a delay in transportation or to sampling error but does not justify the routine practice of taking multiple gastric biopsy specimens for culture purposes. Transportation of specimens took place in sterile saline or Cairy-Blair medium. Although a slightly higher number of positive cultures was obtained with sterile saline as transport medium (table), the difference with Cairy-Blair medium was not significant (p = 0·87). When sterile saline was used as transport medium, 23 *H pylori* positive patients out of 26 patients with evidence of *H pylori* infection (according to the definition) were identified. The use of specialised transport media does not, therefore, seem necessary, and adds only to the costs of transportation. Because of these findings, general availability, and the ease of handling, sterile saline is to be preferred as a transport medium for gastric biopsy specimens. The time for transportation and storage of gastric biopsy specimens at room temperature (20°C) did not significantly (p = 0·64) affect *H pylori* culture results (table). The negative cultures after 24 hours were mainly caused by three negative results in Cairy-Blair transport medium (table), indicating that gastric biopsy speci-

mens can be kept at room temperature in the endoscopy unit and transported to a microbiological laboratory at convenient hours (within 24 hours). We therefore conclude that in previously untreated patients adequate culture results for *H pylori* from gastric biopsy specimens can be obtained by taking one single antral biopsy specimen and by transportation in sterile saline, at room temperature, within 24 hours to a local or regional microbiological laboratory.

We thank Mrs Marga Kersbergen-Kok, Ms JW van Spronsen, and Ms Louise Niepoth for typing the manuscript.

Invaluable help is gratefully acknowledged from the nursing staff of our endoscopy department and the technicians of the laboratory for medical microbiology.

1 Goodwin CS, Armstrong JA, Chilvers T, *et al.* Transfer of Campylobacter pylori and Campylobacter mustelae to *Helicobacter* gen nov. as Helicobacter pylori comb. nov. and Helicobacter mustelae comb. nov. respectively. *Int J Syst Bacteriol* 1989;**39**:397–405.

2 Blaser MJ. Helicobacter pylori and the pathogenesis of gastroduodenal inflammation. *J Infect Dis* 1990;**161**: 626–33.

3 Axon ATR. Helicobacter pylori therapy: Effect on peptic ulcer disease. [Working party report to the world congresses of Gastroenterology]. Sydney. *J Gastroenterol Hepatol* 1991;**103**:131–7.

4 Deltenre M, Glupczynski Y, DePrez C, *et al.* The reliability of urease tests, histology and culture in the diagnosis of Campylobacter pylori infection. *Scand J Gastroenterol* 1989;**24** (suppl 160): 19–24.

5 Barthel JS, Everett ED. Diagnosis of Campylobacter pylori infections: The "Gold Standard" and the alternatives. *Rev Infect Dis* 1990;**12**(S1):107–14.

6 Peña AS, Endtz HPH, Offerhaus GJA, *et al.* Value of serology (ELISA and Immunoblotting) for the diagnosis of Campylobacter pylori infection. *Digestion* 1989;**44**: 131–41.

7 Veenendaal RA, Peña AS, Meyer JL, *et al.* Long term serological surveillance after treatment of Helicobacter pylori infection. *Gut* 1991;**32**:1291–4.

8 McNulty CA, Dent JC. Susceptibility of clinical isolates of Campylobacter pylori to twenty-one. *Eur J Clin Microbiol Infect Dis.* 1988;**31**:566-9.

9 Goodwin CS, Marshall BJ, Blincow ED. 1988. Prevention of nitroimidazole resistance in Campylobacter pylori by coadministration of colloidal bismuth subcitrate: Clinical and in vitro studies. *J Clin Pathol* 1988;**41**: 207–10.

10 Tompkins DS. Survival and growth of Campylobacter pylori. In: Rathbone BJ, Heatly RV, eds. Campylobacter pylori and gastroduodenal disease. Oxford: Blackwell scientific publications, 1989:24–30.