Correspondence

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

Following the study by Veenendaal *et al.*,¹ we attempted to determine the effectiveness of 0.9% saline as a transport medium for *Helicobacter pylori*.

Seventy two consecutive patients (55 men and 17 women; mean age 58 years), attending for diagnostic or follow-up endoscopy, or both, were studied. These patients had duodenal or gastric ulcers, gastroesophageal reflux disease, and "non-ulcer" dyspepsia. Fifty six patients (78%) were receiving H₂-receptor antagonists or proton pump inhibitors, or both. Two biopsy specimens were taken from both the antrum and the corpus. One of the biopsy specimens was then placed in 0.5 ml 0.9% saline and the other in 0.5 ml complex transport medium (brain heart infusion broth). Specimens from the antrum were cultured within three hours, whereas those from the corpus were kept at room temperature for 24 hours before culture. After culture, the remainder of the biopsy specimen was crushed on to a glass slide, air dried, and Gram stained.

Antral biopsy specimens cultured within three hours produced similar culture positive rates when transported in either complex medium (56%) or saline (50%). As expected, there was a higher bacterial yield from antral compared with corpus specimens as *H. pylori* is found predominantly in the former. However, the corpus culture positive rate was particularly low when samples were initially kept in saline for 24 hours (28%) (table).

Culture rates were compared with findings obtained following Gram staining. At three hours, slightly more of the antral biopsy specimens were culture positive than Gram stain positive but at 24 hours, the reverse was true.

Abnormal colony morphology was not seen when specimens were cultured within three hours irrespective of the transport medium. After 24 hours, however, 26% (seven of 27) of isolates transported in complex transport medium demonstrated atypical growth, compared with 85% (17 of 20) of those in saline.

Veenendaal *et al.* excluded all patients who had taken antibiotics, omeprazole, or bismuth containing drugs during the three months prior to the study. However, most of our patients (78%) were taking either H₂-receptor antagonists or proton pump inhibitors. This perhaps is the cause of the

low recovery rates from biopsy specimens transported over 24 hours.

Our investigations show that culture positive rates are similar when biopsy specimens are transported in either saline or complex transport medium provided they are cultured within three hours. In contrast, however, there is a marked reduction in bacterial viability when samples are transported over 24 hours at room temperature, and more so in saline. Strains which survived generally demonstrated uncharacteristic colony morphology. Our data seem to suggest that, provided biopsy specimens can be rapidly transported for culture, the medium used is irrelevant. However, not all hospitals have culture facilities. In such cases delays in biopsy transport are inevitable. With the increased use of proton pump inhibitors, which suppress but do not eradicate *H. pylori*, the viable bacterial load may well be low. To produce the best bacterial yields, we conclude that where delays in culture cannot be avoided, a complex transport medium is required.

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- 1 Veenendaal RA, Lichtendahl-Bernards AT, Peña AS, Endtz HPH, van Boven CPA, Lamers CBHW. Effect of transport medium and transportation time on culture of *Helicobacter pylori* from gastric biopsy specimens. *J Clin Pathol* 1993;46:561-3.

Drs Veenendaal and Bernards comment:

We read the comments of Morton and Bardhan on our study of transport medium and transportation time with great interest. We cannot, however, agree with their conclusion "...that where delays in culture cannot be avoided, a complex transport medium is required".

As stated by Morton and Bardhan, the bacterial yield from antral biopsy specimens is higher than that from corpus specimens. For this reason, antral, and not corpus, biopsy specimens have been generally used for diagnostic purposes. Using antral biopsy specimens, Morton and Bardhan confirmed our finding that there is no difference in diagnostic yield when 0.9% sterile saline is used compared with that obtained using complex media. In their 24 hour study Morton and Bardhan used corpus instead of antral biopsy specimens, which is not common practice, and obtained a lower diagnostic yield. Both the use of corpus biopsy specimens and the fact that patients

with prior treatment for *H. pylori* infection were not excluded make comparisons between the two studies meaningless.

Other investigators have shown that survival of *H. pylori* over 24 hours in saline (0.9%)¹ and results of culture within 24 hours in Stuart's medium were as good as those obtained when the specimens were cultured within two hours,² although refrigeration (4°C) was used in the latter study. When longer transportation or storage time is necessary, specialised transport media (Cysteine-Albimi medium containing 20% glycerol) and a laboratory freezer (-20°C) are probably necessary. This would permit storage for up to four weeks with a claimed recovery rate of 100%.³

We therefore maintain our original conclusion that adequate culture results for *H. pylori* from gastric (antral) biopsy specimens can be obtained within 24 hours following transportation in sterile saline (2 ml, 0.9%) in a small sterile glass jar with screw cap at room temperature.

This protocol is currently in use in a multicentre treatment study, for which culture and determination of antimicrobial susceptibilities are being performed in our laboratory, with good diagnostic results.

- 1 West AP, Millar MR, Tompkins DS. Effect of physical environment on survival of *Helicobacter pylori*. *J Clin Pathol* 1992;45:228-31.
- 2 Kjeller M, Fischer A, Justesen T. Transport conditions and number of biopsies necessary for culture of *Helicobacter pylori*. *Eur J Clin Microbiol Infect Dis* 1991;10:166-7.
- 3 Han SW, Flamm R, Hachem CY, Kim HY, Clarridge JE, Evans DA, *et al.* Transport and storage of gastric biopsies and cultures containing *Helicobacter pylori* [abstract]. *Am J Gastroenterol* 1994;89:1287.

Routine diagnosis of *Pneumocystis carinii* pneumonia

We read the recent paper by Wakefield *et al.*,¹ describing the advantages of DNA amplification in the diagnosis of *Pneumocystis carinii* pneumonia (PCP), with interest. These authors have previously been cautious about the importance of low positive polymerase chain reaction (PCR) results in this infection.² In the three cases studied with granulomatous PCP, however, PCR would have achieved the diagnosis much earlier than current methods and with fewer invasive procedures. The sensitivity required to detect *P. carinii* in bronchoalveolar lavage (BAL) samples from these patients was achieved using a single step PCR followed by Southern blot hybridisation of the PCR product.

We have developed a nested PCR protocol using the same oligonucleotides as Wakefield *et al.* This method only involves further amplification of the single step PCR product by thermal cycling for 20 cycles using an external and internal primer (pAZ102-E of Wakefield *et al.*; sequence: 5'-GATGGCTGTTTCCAAGCCA-3', and pAZ102-L2; sequence: 5'-ATAAGGTAGATAGTCGAAAG-3', respectively). DNA is detected by ethidium bromide staining following agarose gel electrophoresis. This method has three advantages: the assay time is shorter, giving a result within six hours; less technical training is required; and the cost of the test is lower than that of Southern blot hybridisation. These advantages make the nested PCR more suitable

Positivity rate of culture v Gram stain from biopsy specimens transported within three hours and after 24 hours in both saline and complex transport medium

	Transported in complex media		Transported in saline	
	Within three hours (antrum)	After 24 hours (corpus)	Within three hours (antrum)	After 24 hours (corpus)
<i>H. pylori</i> status				
Culture positive	40 (56%)	27 (38%)	36 (50%)	20 (28%)
Gram stain positive	33 (46%)	31 (43%)	32 (44%)	30 (42%)