

# Correspondence

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

Following the study by Veenendaal *et al.*,<sup>1</sup> 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, gastro-oesophageal reflux disease, and "non-ulcer" dyspepsia. Fifty six patients (78%) were receiving H<sub>2</sub>-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 H<sub>2</sub>-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%)<sup>1</sup> 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,<sup>2</sup> although refrigeration (4°) 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%.<sup>3</sup>

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.

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- 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.*,<sup>1</sup> 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.<sup>2</sup> 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%)

for routine use. Our nested PCR gives up to 100 times the sensitivity of the single step PCR, the same amplification achieved by Southern blot hybridisation in the paper by Wakefield *et al.*<sup>1</sup>

We have examined samples from patients with HIV/AIDS and from other immunocompromised patient groups. Although the number of clinical samples tested was small, the results are encouraging. Of 10 HIV respiratory samples, five were positive and five negative on immunofluorescence assay. Identical results were achieved with PCR. However, in seven samples from four immunocompromised patients (non-HIV/AIDS) with none or very few cysts detectable on immunofluorescence assay, PCR was clearly positive. These patients responded to specific antipneumocystis therapy. Immunocompromised patients without HIV/AIDS may have lower numbers of the organism in their respiratory secretions,<sup>3</sup> in which case a sensitive assay has advantages. Our aim was to perfect a rapid sensitive PCR for routine use on respiratory samples, such as BAL or even induced sputum samples which are easier to obtain.

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- 1 Wakefield AE, Miller RJ, Guiver LA, Hopkin JM. Granulomatous Pneumocystis carinii pneumonia: DNA amplification studies on bronchoscopic alveolar lavage samples. *J Clin Pathol* 1994;47:664-6.
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### Taxonomical and epidemiological investigations of *Aeromonas* sp

In their recent article<sup>1</sup> Carey *et al* contend that the universal, commercial 16S + 23S rRNA of *Escherichia coli* is an unsuitable probe for the taxonomical identification of hybridisation groups (groups 1 to 13) of *Aeromonas* sp. For more than two years, we have been studying the taxonomy of *Aeromonas* sp and have examined more than 400 strains originating—for example, from food, water, human fecal samples, and fish (*A. salmonicida*). Ribotyping using universal 16S + 23S rRNA labelled with digoxigenin as the probe has produced reproducible results which are comparable with those reported by Martinetti-Lucchini and Altwegg.<sup>2</sup> For successful identification of hybridisation groups in *Aeromonas* sp, the isolates should have been adequately characterised by conventional methods<sup>3</sup>—procedures used to purify chromosomal DNA are important because extraction of active endonucleases using phenol/chloroform and chloroform is required (polysaccharide contamination can cause problems). The quantity of DNA used is also important. Ribopatterns occurring at molecular weights of about 0.8 to 4 kilobases can be used for the identification of a hybridisation group if DNA is restricted with *Sma*I. For

this area to become visible, 4 to 5 µg of DNA is needed. Carey *et al*<sup>1</sup> report that they used only 1 µg of DNA and I believe that this is why these authors failed to visualise "small" bands. For a molecular weight of 4 to 23 kilobases to be visualised, just 1 µg of DNA is required. This can be used for the epidemiological comparison of related isolates within a hybridisation group, as shown by Martinetti-Lucchini and Altwegg.

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- 1 Carey PE, Eley A, Wilcox MH. Assessment of a chemiluminescent universal probe for taxonomical and epidemiological investigation of *Aeromonas* sp isolates. *J Clin Pathol* 1994;47:642-6.
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- 3 Altwegg M, Steigerwalt AG, Altwegg-Bissig R, Luthy-Ottenstein J, Brenner DJ. Biochemical identification of *Aeromonas* genospecies isolated from humans. *J Clin Microbiol* 1990;28:258-64.

### Drs Carey, Eley and Wilcox comment:

We appreciate the interest shown by Dr Hänninen in our recent publication. In our article we concluded that the universal chemiluminescent probe was unsuitable for taxonomical investigations but was useful for epidemiological studies of *Aeromonas* sp isolates. We have taken note of Dr Hänninen's recommendations and wholeheartedly agree with the first two points raised, which we complied with. It is possible that the use of more than 1 µg of DNA for endonuclease digestion could result in stronger bands in the lower molecular weight (0.8 to 4 kilobases) region. This we were somewhat reluctant to do as at times some of the higher molecular weight bands were quite strong and became difficult to visualise accurately, whilst the low molecular weight bands were hardly visible. Nevertheless, we realise that Dr Hänninen used a digoxigenin labelled probe and this may give a better balance in intensity between the high and low molecular weight bands, permitting the use of more DNA.

### Contamination of crystal violet in the Gram stain method

Crystal violet is used routinely in Gram's method for the staining of bacteria. In our laboratory all stains used in the method are filtered with a grade 113 (30 µm retention) paper filter to remove large stain deposits after preparation. Filtered stains are then stored in Winchester bottles and these are used to refill 500 ml plastic bottles in constant use at the bench.

Recently, Gram negative bacilli were seen in smears stained by Gram's method from a number of samples from normally sterile sites. In most cases clinical features were not suggestive of bacterial infection. All direct cultures were negative on routine media after 48 hours of incubation. The possibility of contamination in one or more stains used in the Gram method was raised.

To identify the source of contamination, a representative sample of the iodine, safranin, and crystal violet solutions were

centrifuged. Deposits from each were placed onto a clean, glass slide with a sterile needle and syringe and then stained by Gram's method using freshly prepared and filtered stains. Numerous Gram negative bacilli were present in the crystal violet deposit only. These grew after enrichment culture and were identified as *Pseudomonas cepacia*. Further investigation revealed that the deionised water used in the preparation of the stain was contaminated with *P cepacia* and *Corynebacterium aquaticum*.

There have been numerous reports of contamination of laboratory reagents; one recent report found the source of contamination to be a phenol red solution.<sup>1</sup> Many different organisms have been implicated in such reports, including *P cepacia*.<sup>2</sup> Contamination of hospital distilled water systems has also been reported.<sup>3</sup> Deionised water is not necessarily bacteriologically sterile and should therefore be filtered with a bacteriological grade filter before use.

In this case the Gram negative bacilli did not present a problem in the interpretation of bacterial culture results. However, their presence on microscopy could be potentially misleading, especially in samples from normally sterile sites such as cerebrospinal fluid.

It is hoped that the results of this investigation highlight the problems associated with the contamination of laboratory reagents. Reagents used for staining or culture techniques in the clinical laboratory should be sterilised and filtered as far as possible using recognised techniques. Containers used to hold laboratory reagents should also be cleaned or replaced frequently.

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- 1 Shears P, Rhodes LE, Syed Q, Watson J. A pseudo-outbreak of tuberculosis. *Communicable Disease Report* 1994;1:R9-10.
- 2 Weinstein RA, Stam WE. Pseudoepidemics in hospital. *Lancet* 1977;ii:862-4.
- 3 Kelly T. Hospital distilled water as source of false-positive acid-fast smears. *Lancet* 1978; i:510.

## Book reviews

**Pneumocystis carinii Pneumonia.** 2nd edn. **Lung Biology in Health and Disease.** Vol 69. (Pp 744; \$195.) Marcel Dekker Inc. 1993. ISBN 0-8247-8854-0.

*Pneumocystis carinii* is among the most common infectious pathogens in humans, and its related disease *P carinii pneumonia* (PCP) is one of the most common opportunistic infections in immunocompromised patients, specially those with AIDS. Before the epidemic of AIDS, only a few researchers were involved in the field of this so-called "enigmatic" pathogen. Peter Walzer heads one of the leading research teams on *P carinii*. The new edition of this book perfectly reflects the tremendous advances made during the