Correspondence

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

Following the study by Veenendaal et al., 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 the gastroscopy clinic, were studied. Of these patients 70% had duodenal or gastric ulcer, gastrooesophageal 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 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 specimens 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 (26%).

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, two (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 laboratories are able to culture H pylori, so it may be useful to standardise transport methods. 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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Dri Veenendaal and Bernards comment:
We read the comments of Morton and Bardhan on our culture findings. 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 limited. To produce the best bacterial yields, we conclude that where delays in culture cannot be avoided, a complex transport medium is required.

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.1 This method only involves further amplification of the single step PCR product by thermal cycling for 20 cycles using an internal and external primer (pAZ102-E of Wakefield et al1; sequence: 5'-GATGCCGTCTTCAAGCAGCC-3', and pAZ102-L2; sequence: 5'-AATAGC- GTAATAGTCAGAACG-3', respectively). DNA is detected by ethidium bromide staining following agarose gel electrophoresis. The methodology was such that 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 a Southern blot hybridisation. These advantages make the nested PCR more suitable with prior treatment for H pylori infection were not included, making comparison between the two studies meaningful.

Other investigators have shown that survival of H pylori over 24 hours in saline (0.9%) and results of culture within 24 hours in Stuart’s medium agreed with those obtained when the specimens were cultured within two hours, although refrigeration (4°C) was used in the latter study. When longer transportation or storage time is necessary, specialised media, (such as Cystine-Albimini 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%.

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 number of other studies, for which culture and determination of antimicrobial susceptibilities are being performed in our laboratory, with good diagnostic results.


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

<table>
<thead>
<tr>
<th>H pylori status</th>
<th>Transformed in complex media</th>
<th>Transformed in saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within three hours (antrum)</td>
<td>After 24 hours (corpus)</td>
<td>Within three hours (antrum)</td>
</tr>
<tr>
<td>Culture positive</td>
<td>40 (56%)</td>
<td>27 (38%)</td>
</tr>
<tr>
<td>Gram stain positive</td>
<td>33 (46%)</td>
<td>31 (43%)</td>
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</tbody>
</table>

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doi: 10.1136/jcp.48.1.91-a

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