Correspondence

Effects of culture media on detection of methicillin resistance in coagulase negative staphylococci

We read with interest the report by Milne et al1 on the effects of culture media on the detection of methicillin resistance in coagulase negative staphylococci (CNS). We agree with their conclusions that the incorporation of salt in Columbia agar is the most reliable culture medium for the detection of methicillin resistance in CNS by disc testing.

The UK National External Assessment Scheme for Microbiology (NEQAS) has shown that generally most laboratories can recognise methicillin resistance in Staphylococcus aureus but have more problems with Staphylococcus epidermidis2 (Snell, personal communication). Some laboratories may secretly underestimating the percentage of isolates of CNS that are resistant to methicillin.

We examined 248 CNS for susceptibility to methicillin by disc testing using three different methodologies. Method 1 used Columbia agar with 2% salt added (CA + 2% salt) whilst method 2 used Mueller Hinton agar containing 2% salt (MH + 2% salt); in both methods incubation was at 35°C for 24 hours. Method 3 incorporated Diagnostic Susceptibility Test (DST) agar plus 1% lysed blood, incubated at 30°C for 21 hours. Strains showing equivocal results were further examined by plate MIC using the three media and respective incubation conditions.

Of the 248 strains tested, 36 were excluded because they were novobiocin resistant and two were Staphylococcus haemolyticus because they were not identified on the ATB 32 Staph system (bioMerieux SA). Of the remaining 210 strains, the MIC results showed 107 (51%) to be resistant to methicillin. The results for the three methodologies are shown in the table.

The method using Columbia agar incorporating 2% salt incubated at 35°C  Methods for determining methicillin sensitivity of staphylococci have undergone development since the first resistant strains were reported, as they were originally developed for S aureus; the same modifications are now being applied for CNS. However, CNS grow less luxuriantly than S aureus and the resistant subpopulation is smaller, making it harder to detect methicillin resistance.3 Where laboratories incorporate salt in their media for methicillin testing, a concentration of 5% is usually used, but this concentration is not well tolerated by staphylococci.4 Previous investigations showed that 2% salt provides an osmotically supportive medium and, in conjunction with Columbia agar, encourages enhanced growth and therefore more clearly demonstrates methicillin resistance. These results are also in general agreement with those of other studies5 and we recommend the use of Columbia agar incorporating 2% salt for the detection of methicillin resistance in CNS.

<table>
<thead>
<tr>
<th>Method</th>
<th>Number of CNS strains</th>
<th>Number of strains sensitive</th>
<th>Number of strains resistant</th>
<th>Showing false resistance</th>
<th>CA + 2% salt</th>
<th>MH + 2% salt</th>
<th>DST ages + 1% lysed blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 107)</td>
<td>(n = 107)</td>
<td>(n = 107)</td>
<td></td>
<td>107 (100%)</td>
<td>93 (87%)</td>
<td>63 (59%)</td>
</tr>
</tbody>
</table>

detected all the resistant strains, but two sensitive strains were identified as resistant. This method is clearly superior to both the other methods tested, although variations of methods based on DST incubated at 30°C appear to be the most common in use in the UK for determining methicillin sensitivity (Snell, personal communication).


Diagnosis of Helicobacter pylori in biopsy specimens

I read with interest the report by Veenendaal et al who showed that a 24-hour delay and storage of antral biopsy specimens in physiological saline solution did not alter the positive culture rate of Helicobacter pylori.1 However, I feel that the definition of the pylori used for the diagnosis of H pylori infection may be misleading, if used without consideration. As they state, it is clear that culture is a 100% specific, though "probably not the most sensitive" test for the identification of H pylori infection. Their case with a positive serology and histology result, but with a negative culture seems to justify this view. Concerning their remaining two positive cases, my opinion is that microscopic identification of an H pylori-like organism in a haematoxylin and eosin stained biopsy specimen alone cannot be accepted as a diagnostic criterion for infection. Haematoxylin and eosin staining is not a generally accepted tool for demonstrating the micro-organisms, which are sufficiently sensitive, though it is ideal for indicating antral gastritis. It is not suitable for detecting early colonisation of the mucosa by the bacterium. Stains such as Wright-Giemsa, Brown-Hopps, or Warthin-Starry silver stain are more commonly accepted for this purpose because they are more sensitive.2 In routine diagnostic work, however, it is advisable to use two of the tests that are discussed in the paper by our colleagues, and mentioned by Nethercutt et al in their discussion.3 One of these may be histological, preferably using special staining methods. Even withdrawal of the two cases where only histology was positive would not influence the result described in the paper.

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Dr J Veenendaal and Lichtendahl-Bernards Cambridge: Although the results of our study as indicated by the correspondents were not influenced by the definition of Helicobacter pylori infection used, their comment addresses an important point.1 There is no general agreement as to which tests should be used as the gold standard in the diagnosis of gastric H pylori infection. In a recent (unpublished) study we found the combination of histology (haematoxylin and eosin staining) and culture as a definition of H pylori infection superior with regard to sensitivity when compared with either test alone (121 patients culture positive, 125 patients positive by histology and 137 patients positive by culture, or both). These findings almost matched the results of a previous validated sensitive and specific enzyme linked immunosorbent assay (ELISA),2 for IgG H pylori antibodies, used in the same population (143 positive patients). Other authors also confirmed the diagnostic value of histology3 and haematoxylin and eosin staining methods.4 However, experience with the test used is essential and here we agree with the correspondents.

In our pathology department there is a long standing interest and experience in the detection of H pylori and its associated gastritis. When in doubt about the diagnosis, especially after treatment when organisms are infrequent or absent in the presence of chronic gastritis, additional staining techniques (Giemsa or Warthin-Starry) are occasionally necessary. This did not apply to our study. We therefore consider our "good standard" appropriate.
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