Correspondence

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

We read with interest the report by Milne *et al*[1] 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 *Staphylococus aureus* but have more problems with *Staphylococcus epidermidis*" (Snell, personal communication). Some laboratories may be seriously 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 Sensitivity 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 more were excluded because they were not identified on the ATB 32 Staph system (bioMerieux SA). Of the remaining 210 stains, 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 detected all the resistant strains, but two sensitive strains were identified as resistant. This medium 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).

Method	*Number of CNS strains resistant to methicillin (n = 107)*	*Number of CNS strains sensitive to methicillin (n = 103)*	*Number of sensitive CNS strains showing false resistance to methicillin*
CA + 2% salt	107 (100%)	101	2
MH + 2% salt	93 (87%)	114	3
DST ages + 1% lysed blood	63 (59%)	147	0

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.[2] Where laboratories incorporate salt in their media for sensitivity testing, a concentration of 5% is usually used, but this concentration is not well tolerated by staphylococci.[3] 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 studies[4] and we recommend the use of Columbia agar incorporating 2% salt for the detection of methicillin resistance in CNS.

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1 Milne LM, Crow MR, Emptage AGM, *et al.* Effects of culture media on detection of methicillin resistance in Staphylococcus aureus and coagulase-negative staphylococci by disc diffusion methods. *J Clin Pathol* 1993;**46**:394–7.
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4 Law D, Megson GM, Keaney MGL, *et al.* The influence of salt concentration on the detection of methicillin resistance in coagulase-negative staphylococci. *J Antimicrob Chemother* 1992;**30**:603–14.

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 they have 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-organism as it is not 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 not fully specific, as mentioned by Neithercut *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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1 Veenendaal RA, Lichtendahl-Bernards AT, Peña AS, *et al.* Effect of transport medium and transportation time on culture of Helicobacter pylori from gastric biopsy specimens. *J Clin Pathol* 1993;**46**:561–3.
2 Madan E, Kemp J, Westblom TU, *et al.* Evaluation of staining methods for identifying Campylobacter pylori. *Am J Clin Pathol* 1988;**90**:450–3.
3 Neithercut WD, Rowe PA, El Nujumi AM, *et al.* Effect of Helicobacter pylori infection on intragastric urea and ammonium concentrations in patients with chronic renal failure. *J Clin Pathol* 1993;**46**:544–7.

Drs Veenendaal and Lichtendahl-Bernards comment:

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 stain) 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 histology or 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 histology[3 4] and haematoxylin and eosin staining methods.[5] 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.

As pointed out in our article, we feel that culture is important for routine diagnostic