Letters to the Editor

Counterimmunoelectrophoresis for the detection of bacterial antigens in cerebrospinal fluid

We read with interest Dr DS Tompkins' article1 comparing Phadebact coagglutination with counterimmunoelectrophoresis (CIE), for the detection of bacterial antigens in cerebrospinal fluid. We would like to draw attention to what may be an important practical point when performing CIE—namely, the dilution of the sample to overcome the problem false negative in the neat sample due to the prozone phenomenon. This point is not specifically mentioned in the ACP Broadsheet on the microbiological examination of cerebrospinal fluid,2 and we wonder if any of your readers have experienced similar cases to the one outlined here.

Case history

A four month old baby girl was admitted with a 24 h history of fever and irritability. No antibiotics had been administered. Investigations included a lumbar puncture, which showed a protein concentration of 1-4 g/l and a cerebrospinal fluid (CSF) glucose concentration that was less than half that of a simultaneous blood glucose measurement. The CSF polymorphonuclear leucocyte count was 180 × 10^6/l, and a number of Gram stains showed a large number of consistently Gram negative cocacobacilli. Some appeared very diplococcioid in morphology, and despite the Gram reaction we considered the possibility of their being pneumococci, a well described phenomenon in fluid culture.3 CIE was performed as described in the Broadsheet 108.2 In view of the large numbers of organisms seen, dilutions of CSF were also tested. All neat specimens were negative when tested against Neisseria meningitidis, Haemophilus influenzae type b, and pneumococcal antiserum, but the pneumococcal test proved positive at dilutions greater than 1/16. Culture performed the next day showed a classic Streptococcus pneumoniae, which unfortunately died before it could be serotyped. The patient responded rapidly to parenteral benzyl penicillin, to which the organism was sensitive.

This case indicates how confusing the Gram stain can be.1 It also highlights the practical importance of the prozone phenomenon. In meningitis treated before lumbar puncture, a large number of bacteria may be lysed and one is unaware of the potential for a prozone. We have so far not assessed the Phadebact coagglutination system, but suspect a prozone may be less of a problem than with CIE.

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References


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Improved blood free selective medium for the isolation of Campylobacter jejuni from faecal specimens

The high incidence of campylobacter enteritis reflects the importance of having available routine isolation facilities and a defined reproducible medium. We have recently described a blood free selective medium—CCD agar—for the isolation of Campylobacter from faeces.1 2 Although this medium gave excellent recovery of some campylobacters, it was less inhibitory than some other selective agars.3 4 The selectivity of the CCD agar was obtained by incorporating cephalin 10 mg/l and sodium deoxycholate 0-1%. In order to improve the selectivity of this medium the cephalo-lin has been replaced with cefoperazone 32 mg/l, an antimicrobial agent that has proved useful in two other recently described campylobacter isolation media.5 6 The modified CCD agar has been compared with the Preston agar7 for the isolation of C jejuni from faecal specimens. Fecal suspensions prepared in 0-1% peptonate water were inoculated on to two plates of each medium using cotton tipped swabs and spread to produce discrete colonies. All plates were incubated microaerobically,7 one pair of each medium at 42°C and the other at 37°C. Plates were examined after 24 h and 48 h incubation, and suspect campylobacter growth was confirmed by the oxidase test and by characteristic cell morphology and motility under dark field microscopy.

Sixty four campylobacter isolations were made from 730 specimens: 62 after incubation at 42°C and 58 after incubation at 37°C (Table). The two specimens which were positive only at 37°C and the six specimens positive only at 42°C produced very scanty growths, indicating that these differences were probably due to sampling. Isolation at both 42°C and 37°C was greater using the modified CCD agar than the Preston agar. Maximum isolation on both media was achieved when plates had been incubated for 48 h.

The modified CCD agar grew contaminating bacteria from only 13.4% of specimens at 42°C and from 25% at 37°C compared with 12.3% and 21.8% respectively on the Preston agar. Moreover, of cultures incubated at 42°C the growth of faecal contaminants outside of the primary inoculum area occurred on only 34% of modified CCD agar plates and 2% of Preston agar plates. The most frequent contaminants to grow on the modified CCD agar were yeasts, but occasionally streptococci and coliforms were present.

The results with the modified CCD agar used at 37°C are encouraging, and when incubation at 42°C is not available the modified medium should produce acceptable recovery of C jejuni from faecal specimens. We believe that in developing

<table>
<thead>
<tr>
<th>Incubation (48 h)</th>
<th>Medium</th>
<th>No of campylobacter isolates</th>
<th>Plates growing faecal contaminants (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>42°C (62)*</td>
<td>Modified CCD agar</td>
<td>60</td>
<td>13-4</td>
</tr>
<tr>
<td></td>
<td>Preston agar</td>
<td>59</td>
<td>12-3</td>
</tr>
<tr>
<td>37°C (58)*</td>
<td>Modified CCD agar</td>
<td>56</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Preston agar</td>
<td>52</td>
<td>21-8</td>
</tr>
</tbody>
</table>

*Total number of isolations at stated temperature.

Growth of campylobacter isolates and contaminants from 730 faecal specimens

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country or in laboratories where the use of blood is a problem and technical expertise is limited the improved selectivity of the blood free medium will simplify the isolation of C. jejuni from faecal specimens.

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References


Batch screening method for the detection of bacteriuria

With regard to the toxic effect of boric acid on bacteria in urine, the percentage of false negatives of 16% quoted by Dr Maskell is inaccurate. The studies referred to were not true comparisons, and the dip slide method used as the reference method is liable to an error of over estimation of about 10%. The percentage of organisms falling into the category of $10^7$ organisms/ml and $>10^8$ organisms/ml mixed culture was 27-8% with the dip slide method, which is an unacceptably high percentage suggesting a poor standard of specimens. The authors concluded that the loss in positivity may be more apparent than real and that it was difficult to show a significant reduction in count in under 24 h.

Our own experience in a laboratory serving several acute hospitals and scattered domiciliary practices is that boric acid preservative solves more problems than it creates and is an excellent preservative for both white and red blood cells.

It is unfortunate that our published method of urine culture could be interpreted as a protocol introducing undue delay; the whole aim of our service in Bury is to provide rapid information for clinical use. We prepare a full plate culture and direct sensitivity on all urines with >10 white blood cells per mm³ and aim to report on quantitative urine bacterial growth with a direct sensitivity test result and presumptive identity of organisms on the morning after the day of receipt of the specimen. This puts out between 85-90% of reports, and where further work is needed an interim report may be issued.

We believe that our approach is a cost effective method of providing rapid clinical reports. In processing over 30 000 urine specimens a year we can have less than a whole time equivalent of scientific officer time being dedicated to urinary work.

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References


The pathology of meconium ileus equivalent

Dr J Jeffrey et al describe sulphomucin in the ileum of adults with cystic fibrosis. They may be interested to learn that Dr S Spicer and I found sulphomucin in duodenum (where normally only mucus and mucus glycocalyx are found) in patients with cystic fibrosis and also in some non-cystic fibrosis patients with duodenal ulcers. It thus seems unlikely that it is unique to that disorder. Moreover, increased sulphomucins are found in other epithelial sites in non-cystic fibrosis inflammatory disorders. Although it is tempting to explain the increased viscosity and other properties of cystic fibrosis mucus on this basis of increased sulphate content, the finding is probably non-specific.

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References


Dr Jeffrey and colleagues comment as follows:

We are grateful to Dr Lev for the interest he has shown in our case report and we are, of course, familiar with his and Dr Spicer’s early publication on the histochemistry of mucus in cystic fibrosis. Their paper was not quoted because, to a large extent, their qualitative work has been confirmed and superseded by the quantitative study of Morrissey and Tymvios, who cited the 1965 article.

We agree with Dr Lev that the presence of sulphomucin in the small intestine is not unique to cystic fibrosis. Indeed, trace amounts of sulphomucin can be detected even in normal small intestinal mucosa (Wells, unpublished observations) though the acidic mucin component is, as we