Letters to the Editor

Counterimmunoelectrophoresis for the detection of bacterial antigens in cerebrospinal fluid

We read with interest Dr DS Tompkins' article[1] 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 potential 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 coccobacilli. Some appeared very diplococcoid 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 antisera, 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.

B COOKSON*
A BALDWIN
Department of Microbiology, North Middlesex Hospital, London N18 1QX

References

[1] Tompkins DS. Comparison of Phadebact coagglutination tests with counterimmunoelectrophoresis for the detection of bacterial antigens in cerebrospinal fluid. *J Clin Pathol* 1983;**36**:819–22.
[2] Fallon RJ. Microbiological examination of cerebrospinal fluid. *ACP Broadsheet 108,* 1983.
[3] Adeniyi-Jones C, Stevens DL, Rasquinha ES. False no-growth blood cultures in pneumococcal pneumonia. *J Clin Microbiol* 1980;**12**:572–5.

*Present address: Department of Microbiology, St Thomas' Hospital, London SE1 7EH.

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 campylobacters, it was less inhibitory than some other selective agars.[3,4] The selectivity of the CCD agar was obtained by incorporating cephazolin 10 mg/l and sodium deoxycholate 0·1%. In order to improve the selectivity of this medium the cephazolin 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 agar[4] for the isolation of *C jejuni* from faecal specimens. Faecal suspensions prepared in 0·1% peptone 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,[1] 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 inubation 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 contaminants 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 3·4% 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

Growth of campylobacter isolates and contaminants from 730 faecal specimens

Incubation (48 h)	*Medium*	*No of campylobacter isolates*	*Plates growing faecal contaminants (%)*
42°C (62)*	Modified CCD agar	60	13·4
	Preston agar	59	12·3
37°C (58)*	Modified CCD agar	56	25
	Preston agar	52	21·8

*Total number of isolations at stated temperature.

countries 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.

DN HUTCHINSON
FJ BOLTON
Public Health Laboratory, Preston Infirmary, Preston PR1 6PS

References

1 Bolton FJ, Hutchinson DN, Coates D. Blood free selective medium for isolation of *C jejuni* from faeces. *J Clin Microbiol* 1984;**19**:169–71.
2 Hutchinson DN, Bolton FJ. Is enrichment culture necessary for the isolation of *Campylobacter jejuni* from faeces? *J Clin Pathol* 1983;**36**:1350–2.
3 Lauwers S, De Boeck M, Butzler JP. Campylobacter enteritis in Brussels. *Lancet* 1978;i:604–5.
4 Bolton FJ, Robertson L. A selective medium for isolating *Campylobacter jejuni/coli*. *J Clin Pathol* 1982;**35**:462–7.
5 Martin WT, Patton CM, Morris GK, Potter ME, Puhr ND. Selective enrichment broth medium for isolation of *Campylobacter jejuni*. *J Clin Microbiol* 1983;**17**:853–5.
6 Butzler JP, De Boeck M, Goosens H. New selective medium for isolation of *Campylobacter jejuni* from faecal specimens. *Lancet* 1983;i:818.

Batch screening method for the detection of bacteriuria

With reference to the comments made by Dr Maskell[1] concerning our published work,[2] we believe that certain points need clarification.

We support the opinion that it is not feasible to pass a midstream specimen of urine into the narrow necked 5 ml boric acid container, in which we receive our specimens; but this objection also applies to the 30 ml universal size container. Wide necked containers of various forms are recommended for collecting midstream specimens,[3] and some containers such as "honey jars" are expensive to recycle. Our solution is to use disposable 200 ml paper cups, referred to by the Americans as "Dixie cups," which cost less than one pence each. When gently squeezed the cups fold to form a delicate pouring spout. The lack of absolute sterility does not affect the culture result in midstream specimens, although they should not be used where catheter or ureteric urine is being collected.

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 10^7–10^8 organisms/l and $>10^8$ organisms/l 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 mm^3 and aim to report on quantitative urine bacterial growth with a direct sensitivity test result and presumptive identity of organisms[4] 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.

PETER KERFOOT
DUNCAN McGHIE
Clinical Microbiology Department, Bury General Hospital, Bury, Lancs BL9 6PG

References

1 Maskell R. Batch screening method for the detection of bacteriuria. (Letter). *J Clin Pathol* 1984;**37**:355–6.
2 Kerfoot P, McGhie D, Cahill E, Fountain T. A mechanised batch screening method for the detection of bacteriuria. *J Clin Pathol* 1983;**36**:1318–9.
3 Meers PD, ed. *The bacteriological examination of urine: report of a workshop on needs and methods.* Public Health Laboratory Service. Monograph Series No 10. London: HMSO, 1978:75–83.
4 Kerfoot P, McGhie D. An empirical approach to the identification of *Escherichia coli* from urines. *Communicable Diseases Scotland* 1983;**17**:12.

The pathology of meconium ileus equivalent

Dr I Jeffrey *et al*[1] 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 sialomucins reside) in patients with cystic fibrosis and also in non-cystic fibrosis patients with duodenal ulcers.[2] 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.

ROBERT LEV
Department of Pathology, Roger Williams General Hospital, Providence, Rhode Island 02908, USA

References

1 Jeffrey I, Durrans D, Wells M, Fox H. The pathology of meconium ileus equivalent. *J Clin Pathol* 1983;**36**:1292–7.
2 Lev R, Spicer S. A histochemical comparison of human epithelial mucins in normal and in hypersecretory states including pancreatic cystic fibrosis. *Am J Pathol* 1965;**46**:23–47.

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 Tymvois,[1] who cite 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