Technical method

A selective enrichment broth for the isolation of *Clostridium difficile*

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The cycloserine, cefoxitin, fructose agar medium (CCF agar) devised by George *et al*[1] and the modification of it where egg yolk is replaced by horse blood (Oxoid CCF agar), have been widely and successfully used for the isolation of *Clostridium difficile* from faecal specimens.[1-4] For epidemiological and environmental work where only small numbers of organisms may be present, it is likely that a more sensitive method of culture is required. Hafiz *et al* used a broth containing *p*-cresol in studying vaginal carriage of *C difficile*[5] whilst Wilson *et al*[6] have reported that the incorporation of sodium taurocholate in CCF agar (in place of egg yolk) enhances the recovery of spore forms of *C difficile* from solid media. We describe the use of cycloserine cefoxitin fructose broth containing 0·1% sodium taurocholate (CCFT broth) for the isolation of *C difficile* from vaginal and faecal specimens.

Material and methods

MEDIA

Solid media

1 Reinforced clostridial agar (RCA, Oxoid) supplemented with 0·2% *p*-cresol (BDH)—group II patients only.
2 Cycloserine cefoxitin fructose blood agar (CCF agar Oxoid).

Enrichment broth

The broth was made up in our laboratory to the same formula as the commercial CCF medium (Oxoid), but omitting the agar and including 1 g/l of sodium taurocholate.

PATIENTS

Five groups of subjects were studied independently (Table). Groups I–III were unselected consecutive women attending the Department of Genital Medicine (DGM). Groups IVa and IVb were mothers attending the maternity unit. Predelivery high vaginal swabs were taken by the midwife at onset of labour. Post delivery vaginal swabs were taken just prior to discharge. Group V were the babies delivered to mothers in group IV.

Culture method

Swabs were inoculated onto solid media and then discarded. When enrichment cultures were performed (see Table), a duplicate swab was broken off into the CCFT broth.

Cultures were incubated in an anaerobic cabinet (10% CO_2) for 7 days. Plates were examined for typical *C difficile* colonies after 48 h and seven days. Suspect colonies were subcultured to blood agar for "purity" prior to further tests.

ENRICHMENT

CCFT broths were subcultured to blood agar after 48 h and seven days. Suspect colonies on blood agar after 48 h were streaked out for purity and treated as for the direct plate subcultures. Isolates were identified as *C difficile* by their typical colonial and Gram stain morphology, distinctive odour and pattern of volatile fatty acid production as detected by gas liquid chromatography.[7]

Results and discussion

High vaginal swabs

The results are summarised in the Table. Only one *C difficile* isolation was made from 132 high vaginal swabs examined using CCF agar in groups I and II. No positive isolates were obtained using the RCA medium containing *p*-cresol (group II). However the use of CCFT broth greatly increased the isolation rates from vaginal swabs. In groups III and IVa the isolation rates from broth were 11% and 18% respectively compared with 1·2% and 0% from CCF agar alone. These differences in isolation rates are significant ($p < 0·01$). In group IVb (post-delivery mothers) CCFT broth was again superior to CCF agar but the total numbers are small and not statistically significant. However, when the overall isolation rates from vaginal specimens are examined for groups II, III and IV combined, the rate of isolation from CCFT 22/177 (12%) is significantly greater than that from CCF agar 3/177 ($p < 0·001$).

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Isolation rates of C difficile in five groups of subjects

Group	No of subjects	Description of subjects	Specimen	Media	Isolation rate
I	62	Consecutive women attending DGM*	Single HVS	CCF agar	0/82
II	90	Consecutive women attending DGM*	Single HVS	CCF agar RCA + 0·1% p-cresol	1/50 0/50
III	82	Consecutive women attending DGM*	Duplicate HVS	CCF agar CCF broth	1/82 (1·2%) p < 0·01 9/82 (11%)
IV (a)	90	Mothers predelivery	Duplicate HVS	CCG agar CCF broth	0/50 (0%) p < 0·0027 9/50 (18%)
IV (b)	45	Mothers postdelivery	Duplicate HVS	CCF agar	2/45 (4·4%) 4/45 (8·8%)
V	50	Neonates 2nd–5th day	Swab from rectum or soiled nappy	CCF agar CCF broth	29/50 (58%) 28/50 (56%)

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p was derived from standard error (SE) using *SE of differences in proportion* = $\sqrt{\frac{p(100-p)}{n1} + \frac{p(100-p)}{n2}}$
HVS = high vaginal swab.

NEONATAL STOOL SPECIMENS (GROUP V)
In contrast to the results obtained with vaginal swabs, the isolation rate obtained using CCFT broth was not significantly different to that obtained using CCF agar. This may be explained by the relatively high counts of *C difficile* organisms present in many faecal specimens and by the occasional failure of CCFT broth to yield *C difficile* when overgrowth of "coliform" organisms had taken place in the broth.

In conclusion we suggest that a liquid culture medium, such as that described here, is a useful addition to conventional agar culture when only small numbers of *C difficile* organisms are likely to be present.

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Rapid demonstration of nucleic acids using "oxidised" gallocyanin and chromic potassium sulphate: methods and applications

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Studies of metallic salt lakes of the oxazine dyes gallamin blue, celestine blue and gallocyanin led to the introduction of techniques for selective staining of Nissl and nuclear substance in nerve cells[1] and specific nuclear stains[2–4] and to the theory of gallocyanin chromalum staining and its application for quantitative estimation of basophilia.[5]

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The gallocyanin chromalum technique of Einarson[1] was reviewed by us for use with the Quantimet image analysing computer[6] and has now been adopted as a nuclear stain for the automated interactive cervical cancer screening system CERVIFIP where detection by integrated optical density is utilised, but its use in routine service conditions is limited by the fact that the staining technique requires incubation at 42°C for 16 h.[7]

We have therefore conducted experiments to reduce the staining time required to a minimum but still retain its degree of stoichiometry and stain density. Our results have also shown that the rapid staining technique is useful for staining cells other than those exfoliated from the cervix, namely