ISOLATION OF *CLOSTRIDIUM DIFFICILE* FROM FAECAL SPECIMENS – A COMPARISON OF CHROMID C. DIFFICILE AGAR AND CYCLOSERINE CEFOXITIN FRUCTOSE AGAR (CCFA) CONTAINING TAUROCHOLOATE

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Introduction: : In the past 10 years the epidemiology of *Clostridium difficile* infection (CDI) has undergone great change with the arrival and rapid dissemination of epidemic strains (2,7). This has resulted in more severe disease (1, 4, 6), and highlighted the requirement for improved and continuing surveillance and the on-going need for stool culturing to facilitate molecular tracking to monitor outbreaks and follow the spread of particular strains and ribotypes (3,4). Here we compared bioMérieux ChromID Cdiff (CDIF) to pre-reduced CCFA with 1% sodium taurocholate (TCCFA) for the isolation of *C. difficile* using direct culture and alcohol shock with glutamate dehydrogenase (GDH) positive faecal specimens that were either PCR-positive or negative.

Methods: All specimens used were GDH positive and in total150 GeneOhm positive samples and 84 GeneOhm negative samples were collected. For direct culture 50 GeneOhm positive stools were collected and stored at -70°C until used. All liquid stools were plated undiluted (25 µl) and solid stools were diluted 1:2 in sterile saline before plating (50 µl). Growth of *C. difficile* and other faecal flora were recorded semi-quantitatively as 1, 2 or 3. For alcohol shocks 100 GeneOhm positive samples and 84 negative samples were tested. Solid stool specimens (1 g) were mixed with 1 ml of ethanol; for liquid stools 1 ml was mixed with 1 ml of ethanol; and for rectal swabs 1 ml of ethanol was added. The faecal material was left in contact with the ethanol for 60 min and then vortexed and 10 µl plated out onto CDIF and pre-reduced TCCFA. The GeneOhm positive faecal samples contained 36 stools (17 liquid) and 64 rectal swabs and the GeneOhm negative samples 36 stools (19 liquid) and 48 rectal swabs. All plates were incubated in an anaerobic chamber and read at both 24 h and 48 h with no plate >15 min outside the chamber during examination and/or manipulation. Presumptive *C. difficile* colonies were sub-cultured onto blood agar for further identification and PCR used to detect toxin A, toxin B and binary toxin genes. Isolates from alcohol shocks were also PCR ribotyped.

Table 1. Sensitivity and recovery of *C. difficile* on CDIF and TCCFA from GeneOhm positive faecal specimens

GeneOhm positive	Sensitivity %	Recovery %
Direct Culture CDIF	100 (47/47)	94 (47/50)
Alcohol shock CDIF	99 (95/96)	95 (95/100)
Direct Culture TCCFA	87 (41/47)	82 (41/40)
Alcohol shock TCCFA	96 (92/96)	92 (92/100)

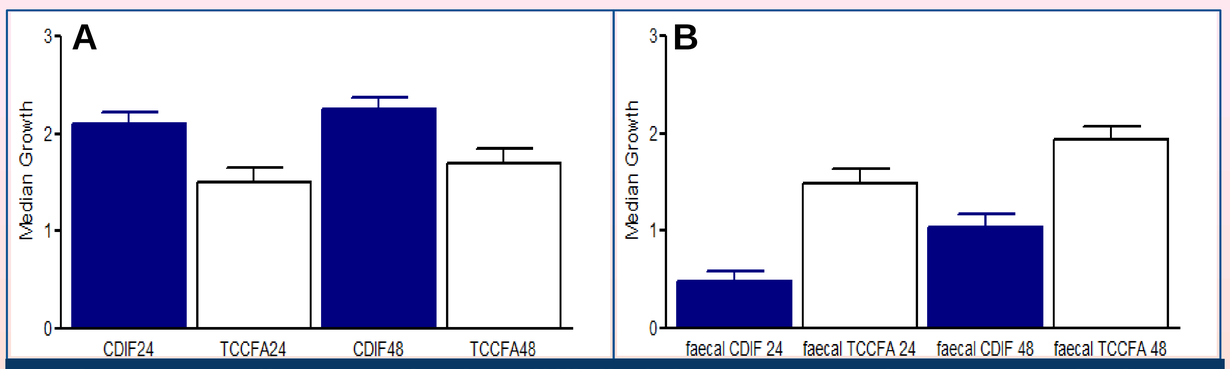

Figure 1. Median growth of *C. difficile* (A) on CDIF and TCCFA and other faecal flora (B) from GeneOhm positive stools using direct culture at 24 h and 48 h

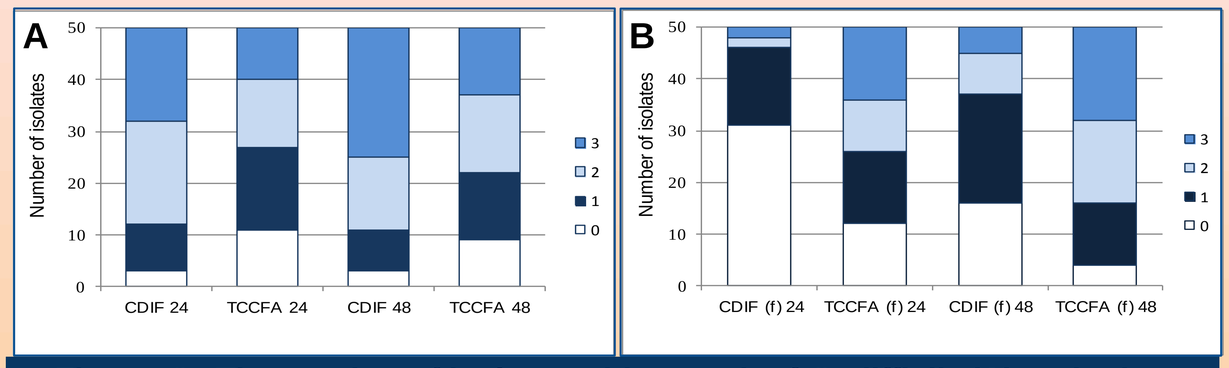

Figure 2. The number of isolates with growth of *C. difficile* (A) and other faecal flora (B) semi-quantified as 1, 2 or 3 from GeneOhm positive stools using direct culture at 24 h and 48 h

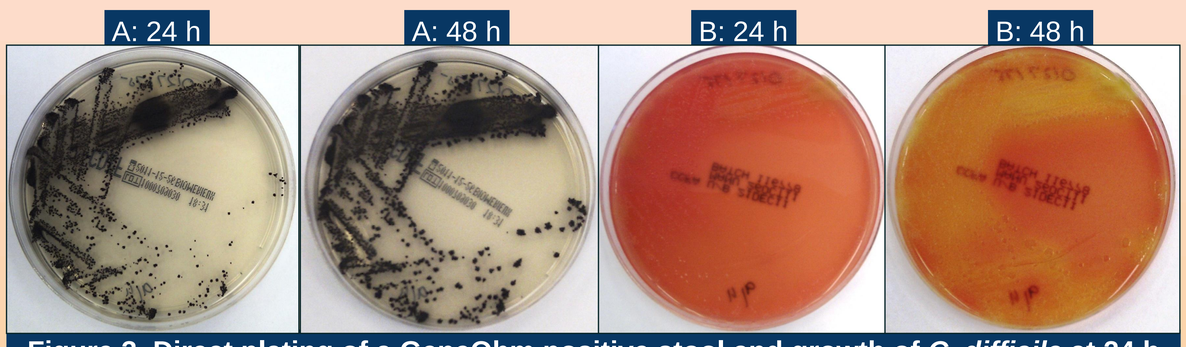

Figure 3. Direct plating of a GeneOhm positive stool and growth of *C. difficile* at 24 h and 48 h on CDIF (A) and TCCFA (B)

Results: Direct culture and alcohol shock of GeneOhm positive faecal samples on CDIF gave similar sensitivity and recovery for *C. difficile* (Table 1). Alcohol shocks of these samples plated onto TCCFA had similar values for sensitivity and recovery which were 10% higher than that for direct culture on the same medium (Table 1).

With direct plating, there was a significant difference between growth of *C. difficile* on CDIF at 24 h and TCCFA at 48 h (*p* 0.007) and between the two media at 48 h ($p < 0.001$) (Figure 1). The difference between growth of *C. difficile* on CDIF at 24 h and 48 h was also significant (*p* 0.0068) (Figure 1). The growth of other fecal flora on CDIF at 24 h and 48 h was significantly different from that on TCCFA at the same time points ($p<0.0001$ at 24 h and 48 h) (Figure 1). Contingency tables were constructed and analysed by the Chi squared test for independence. CDIF grew more *C. difficile* than did pre-reduced TCCFA (24 h *p* 0.0162 and 48 h *p* 0.0457) and less faecal flora (24 h $p < 0.0001$ and 48 h *p* 0.0002) (Figures 2 & 3). *C. difficile* isolated after alcohol shock from the 84 GeneOhm negative faecal samples grew on 41 CDIF plates at 24 h and 36 TCCFA plates at 48 h. When these results were analysed by Fisher's Exact Test no significance difference was found between the two media.

A total of 96 isolates of *C. difficile* were recovered after alcohol shock from the 100 GeneOhm positive samples including 81 that were $A^+B^+CDT^-$, 5 that were $A^+B^+CDT^+$ and 10 that were non-toxigenic. The isolation of non-toxigenic *C. difficile* from GeneOhm positive samples infers that these patients had mixed infections. Four of the non-toxigenic isolates were UK ribotype 010. The toxigenic isolates belonged to several different ribotypes; 014 (n=24), 002 (n=13), 020 (n=7), 012 (n=2), 001 (n=1) and 015 (n=1). For the other 38, including the $A^+B^+CDT^+$ isolates, a ribotype could not be assigned. Among the 46 *C. difficile* isolates from the GeneOhm negative samples 11 were $A^+B^+CDT^-$ and one was $A^-B^+CDT^-$. Four $A^+B^+CDT^-$ isolates were typed as 020 and one was 014. For the other 6 and the $A^-B^+CDT^-$ strain, a ribotype could not be assigned. Of the 34 non-toxigenic isolates 8 were 010.

CONCLUSION

- CDIF with direct plating had similar sensitivity and recovery to both CDIF and TCCFA with alcohol shock
- CDIF was more selective than TCCFA with more growth of *C. difficile* and less contaminating faecal flora
- CDIF grew *C. difficile* in 24 h

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