Detection of Asymptomatic *Clostridium difficile* Carriage by an Alcohol Shock Procedure

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Direct inoculation to cefoxitin-cycloserine-fructose agar and broth was compared with alcohol shock-chopped meat broth inoculation for optimal detection of *Clostridium difficile* in fecal samples. Alcohol shock is significantly more sensitive than cefoxitin-cycloserine-fructose agar or broth and may be the method of choice to detect *C. difficile* in asymptomatic carriers.

The etiologic link between *Clostridium difficile* and pseudomembranous colitis has been well documented (1, 2). Asymptomatic carriers of *C. difficile* may serve as potential sources of organism spread to these susceptible hospital patients, or carriers may themselves become clinically ill following antibiotic treatment. This study was designed to obtain an optimal culture method for detection of low numbers of *C. difficile* in patients without clinical illness. Serial 1:10 dilutions made of *C. difficile* grown for 48 h in chopped meat broth (CMB) adjusted to a 0.5 McFarland standard were used to seed a *C. difficile*-negative stool specimen. All media and diluents were reduced overnight in an anaerobic chamber before use. Stool samples (0.3 g) suspended in 0.9 ml of nonreduced sterile saline were seeded with 0.1 ml of 1:10^2, 1:10^3, 1:10^4, and 1:10^5 dilutions. Cefoxitin-cycloserine-fructose agar (CCFA; Dimed, St. Paul, Minn.) and cefoxitin-cycloserine-fructose broth (CCFB) (CCFA without agar) media (3) were inoculated with each dilution in duplicate. Alcohol shock was performed aerobically on duplicate serial dilutions mixed 1:1 with 95% ethanol and agitated for 45 min. The samples were aerobically washed twice in sterile saline at 2,000 × g for 15 min and added under anaerobic conditions to prerduced CMB (Anaerobe Systems, San Jose, Calif.). All media were incubated anaerobically for 48 h at 35°C. CCFB was subcultured to CCFA and brucella base blood agar (Ana-BAP; Dimed), and CMB was subcultured to CCFA. *C. difficile* was identified by characteristic morphology macroscopically and microscopically on CCFA and brucella agar. Gas-liquid chromatography was performed on all questionable isolates.

In a preliminary study, 107 stool samples collected from patients and submitted to the clinical microbiology laboratory of the Veterans Administration Medical Center in Minneapolis, Minn., for *C. difficile* culture were refrigerated at 4°C, batched, and analyzed by the three different culture methods 1 to 7 days after collection. Stool samples from patients were inoculated onto CCFA and CCFB, alcohol shocked (0.3 g of stool was suspended in 1.0 ml saline), and processed as were the above-described seeded stools. Specimens were also directly plated onto CCFA by the clinical diagnostic laboratory within hours of receipt of the stool specimen.

During a 9-month prospective study on a ward with a history of *C. difficile* outbreaks, rectal swabs and stools were obtained from patients on a weekly basis. Rectal swabs were plated only to CCFA, and the stools were directly plated to CCFA and processed by the alcohol shock method. Statistics were performed by the chi-square method.

In preliminary experiments, *C. difficile* was detected by the CCFA and CCFB methods at 100 colonies per ml and by the alcohol shock method at 1.0 colony per ml. Of 107 stool samples, 19 (18%) were positive for *C. difficile* by direct immediate plating to CCFA. After refrigerated storage for 1 to 7 days, 25 were positive by a combination of all three methods. Of these 25 *C. difficile*-positive cultures, 24 were found to be positive by the alcohol shock method, 21 were positive by CCFB, and 16 were positive by CCFA. No significant difference was found between CCFA and alcohol shock (0.05 < P < 0.1). Three positive specimens found by immediate direct plating of stools to CCFA were subsequently negative by direct CCFA plating after 1 to 7 days of storage. The alcohol shock method detected *C. difficile* in all three of these stored specimens.

In the prospective stool and rectal swab carrier surveillance study, 1,156 rectal swabs were obtained from 630 patients; 464 swab specimens were unpaired, and 692 were paired to stools received from patients within 4 days of the rectal swab culture. Of 692 stool samples, 158 (23%) were positive by alcohol shock compared with 98 of 692 (15%; P < 0.005) by stool plated directly on CCFA and 84 of 692 (13%; P < 0.005) by paired rectal swab plated directly on CCFA. Of 464 unpaired rectal swabs, 57 (12%) were positive; this is comparable to the 13% positivity rate of the paired rectal swab specimens. Of 177 positive specimens in the paired comparison, 66 were positive only by alcohol shock, 65 were positive by all three methods, and 21 were positive by direct plating of stool to CCFA and by alcohol shock. The sensitivities of the three procedures were as follows: alcohol shock, 158 of 177 (89%); direct CCFA stool plating, 98 of 177 (55%); rectal swab, 84 of 177 (47%). The false-negative culture rates were 11, 45, and 53%, respectively.

Of 77 patients in the preliminary study, 7 were considered to have *C. difficile*-associated diarrhea by the definition of six or more unformed stools in 36 h (4). All of these patients had *C. difficile* detected by alcohol shock, CCFA, and CCFB culturing. A significantly larger number of *C. difficile*-positive cultures was obtained with the alcohol shock method than with the conventional method of direct stool plating to CCFA.
Several factors may have led to this increased yield, including culture of a larger sample volume, subsequent growth of the inoculum in the enrichment CMB, and reduction of competitive vegetative organisms by alcohol. Levett and Margaritis-Bassoulis found that the \textit{C. difficile} isolation rate was significantly higher in alcohol-shocked samples grown in CMB for 5 days versus alcohol-shock samples directly subcultured to CCFA (6). Levett, in a similar experiment, found a 50% increase in positive cultures by enrichment culture techniques (with and without alcohol shock) over direct plating to CCFA (5).

Patients with diarrhea may have sufficiently large numbers of organisms to enable consistent detection by standard methods, such as direct plating to CCFA. We do not feel that our data justify the added time and work of the alcohol shock procedure in the processing of diarrhea stool specimens.

The possibility of skewed results due to specimen age was a concern in our pilot project using specimens 1 to 7 days old, but stools from the surveillance study which were cultured without prior storage also demonstrated the superior sensitivity of the alcohol shock method. The use of rectal swabs for carrier detection remains an important method for epidemiologic study of asymptomatic carriers, because samples can be obtained immediately and without an available stool specimen (7). We believe that alcohol shock is sufficiently more sensitive than the other methods tested to warrant its continued use in the detection of \textit{C. difficile} in stool specimens from asymptomatic carriers.

**LITERATURE CITED**