COMPARISON OF BROTH MEDIA FOR RECOVERY OF *CLOSTRIDIOIDES DIFFICILE* FROM ENVIRONMENTAL SAMPLES

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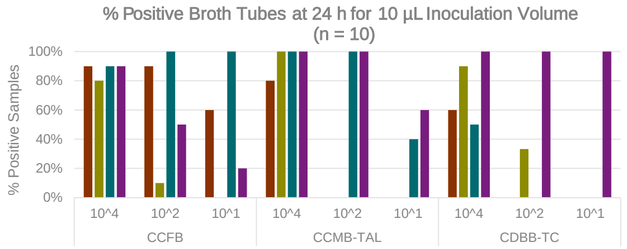
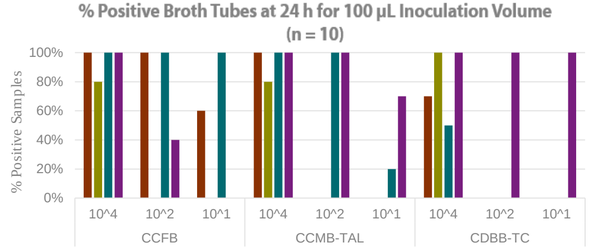
INTRODUCTION

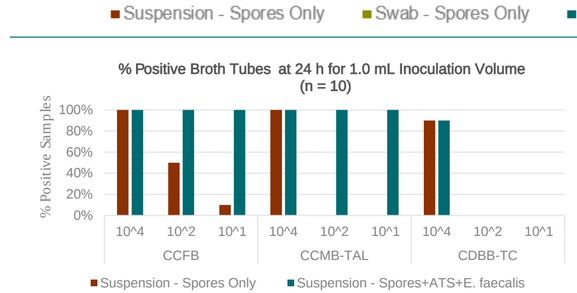
Clostridioides difficile is the most common cause of healthcare-associated diarrhea; in 2011 the burden was estimated at 453,000 infections and 29,000 deaths with 65.8% of the infections being considered healthcare-associated and 24.2% of onset occurring during hospitalization (Lessa et al., 2015). Most patients remain asymptomatic after infection but often continue to shed *C. difficile* in their stool. In the healthcare environment this can be cause for concern since *C. difficile* spores can persist in the environment for long periods of time. Contamination of the environment, especially high-touch surfaces in patient bathrooms and rooms can be sources of infection.

In a recent study to determine bioburden on high-touch healthcare environmental surfaces *C. difficile* was recovered by culture from 15.7% of cleaned rooms (Shams, 2016). While the range was low 64.7 - ≤ 1 CFU/100 cm^2 the infectious dose of *C. difficile* is unknown and the presence of live spores in a patient's environment after cleaning is cause for concern. Current methods to quickly identify *C. difficile* involve use of PCR, however, PCR can only identify the presence of *C. difficile*, and is not capable of distinguishing if the DNA is from live or dead cells. This distinction is critical when assessing the threat to a patient's health. Since healthcare environments are constantly being cleaned and/or decontaminated the likelihood of obtaining dead organisms in an environmental sample is great. Use of broth media to confirm the presence or absence of live *C. difficile* spores could be used as a rapid and sensitive method to monitor room cleanliness and assess contamination during an outbreak situation. Use of anaerobic media can be a challenge since special equipment is needed to incubate the samples. The purpose of this study was to compare two anaerobic broths, Cycloserine cefoxitin fructose broth (CCFB) and Cycloserine cefoxitin mannitol broth with taurocholic acid lysozyme and cysteine (CCMB-TAL), and an aerobically incubated broth Banana Broth (CDBB-TC). Inoculation method (suspension vs. swab) and sample volume were both assessed as different surface sampling tools (wipes or swabs) have different volumes of end product to culture.

RESULTS





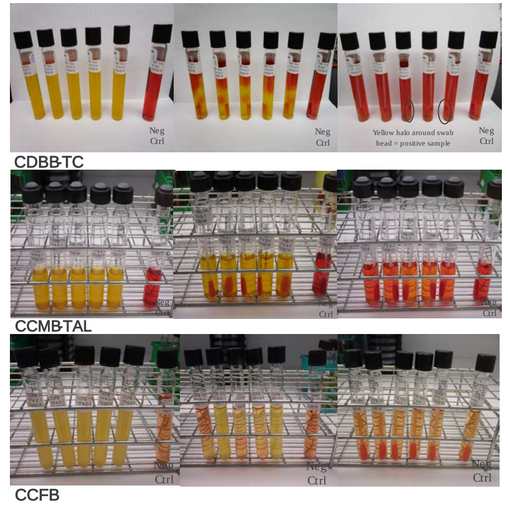


Spores + ATS

NOTE: When spores+ATS was added to broth, the inoculum suspension was a $\log_{10}$ lower than expected and thus only 10^1 spores/mL inoculum is directly comparable to the other sample matrices.

- At 24 h more broth tubes, for all 3 media, were positive than spores alone but fewer were positive than spores+ATS+*E. faecalis*, although the difference was not significant (p>0.05) for all comparisons.
- CDBB-TC inoculated with a swab had higher % positives at 10^1 than CCFB and CCMB-TAL.
- At 48 and 72 h almost all broth tubes for all inoculum types and volumes were 100% positive (except possible true negatives).

24 Hours



METHODS

Media:
CCFB – 5 mL (pre-reduced overnight)
CCMB-TAL – 5 mL (Anaerobe Systems)
CDBB-TC – 10 mL (Hardy Diagnostics)

Organisms:
Clostridioides difficile spores ATCC 9689
Enterococcus faecalis ATCC 29212

Swabs: Puritan HydraFlock® Swabs
20% Artificial Test Soil (ATS) – old formula (Healthmark Industries)

1. A known spore stock (10^8/10^7 spores/mL) was serial diluted down to 10^1 spores/mL
2. Three different inoculum matrices were evaluated at three different inoculated spore concentrations (~10^4, 10^2, and 10^1 spores/mL):
   - Spores only in Phosphate buffered saline with 0.02% Tween 80® (PBST)
   - Spores mixed with 20% ATS
   - Spores mixed with 20% ATS and 10^4 CFU/mL *E. faecalis*
3. Two different inoculation methods (direct addition of the spore suspension and spiking of a flocked swab) and 3 different volumes (10µL, 100µL, and 1mL) were evaluated.
   - 1 mL spore suspension was only directly added to the broth.
   - There were 5 replicates per run, 2 runs total for each spore concentration, inoculation volume, and inoculation method.
   - CCFB and CCMB-TAL tubes were vortex after addition of the suspension or swab while CDBB-TC tubes were inverted twice to mix.
4. There was one negative control (PBST) for each media, inoculation type, and inoculum level.
5. CCFB and CCMB-TAL samples were incubated in an Anaerobic Chamber at 35°C while CDBB-TC samples were incubated in an aerobic incubator at 35°C.
6. Sample results were recorded at 24, 48, and 72 hours.
7. Student's t-test used to compare significance of percent positives (p≤0.05).

CONCLUSIONS

- At 24 h:
  - Spores Only: positive results varied but overall CCFB and CCMB-TAL had more positive broth tubes than CDBB-TC.
  - Spores+ATS+*E. faecalis*: CDBB-TC was equivalent to or higher than CCFB and CCMB-TAL when a spiked swab was used to inoculate the broth.
  - When 1 mL eluent was placed in broth, the anaerobically incubated media were more often positive.
- By 48 h almost all samples were positive for all three broths, inoculation methods, volumes and concentrations.
- At 72 h all samples (except possible true negatives) were positive with a LOD of 10^1 spores/mL.
- Media, inoculation method, and volume of sample are equal within 72 h no matter what the sample matrix was and thus which method to use to test environmental samples may be more dependent on lab capacity.
- The option to do environmental sampling and broth culture is available to labs with and without an anaerobe chamber.

National Center for Emerging and Zoonotic Infectious Diseases
Division of Healthcare Quality Promotion