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BRIEF COMMUNICATION

Isolation and quantitation of *Clostridium difficile* in aqueous and fecal matter using two types of selective media



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Abstract We evaluated the isolation and quantitation of *Clostridium difficile* from aqueous and fecal samples utilizing ChromID CDIF and cycloserine, cefoxitin, and fructose-containing agar with horse blood and taurocholate media. Growth was similar between the two. ChromID CDIF provided enhanced isolation and required no ethanol pretreatment to inhibit normal flora. ChromID CDIF also improved turn-around time, requiring only 24 hours' incubation. Copyright © 2015, Taiwan Society of Microbiology. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

The prevalence of *Clostridium difficile* infections (CDI) has increased dramatically over the past decade.¹ The methods used to identify the presence of *C. difficile* in the clinical microbiology laboratory have also increased. The gold standard continues to be culture on cycloserine, cefoxitin, and fructose-containing agar (CCFA) followed by cytotoxin detection. Other diagnostic testing mechanisms include

enzyme immunoassay and polymerase chain reaction (PCR) testing. While each diagnostic has its pros and cons, a full review can be found in the Society for Healthcare Epidemiology of America/Infectious Diseases Society of America *C. difficile* guidelines.² ChromID CDIF, a new chromogenic agar developed by bioMérieux (Marcy l'Etoile, France) has provided another way to culture and identify *C. difficile*. Previous studies utilizing this media have identified enhanced recovery of *C. difficile* from fecal samples on ChromID CDIF compared with other media types.^{3,4} A recent publication by Luk et al⁵ also demonstrated a beneficial cost reduction using chromogenic agar and a

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slight increase in identification of positive specimens (9%) compared with real-time PCR. This was in exchange for an increase in turn-around time of 1 day. While the isolation of *C. difficile* using different plates has been extensively described, the quantitation of *C. difficile* within the stool of infected patients has not. As such, we aimed to quantitatively and qualitatively assess the performance of ChromID CDIF and CCFA with horse blood and taurocholate (CCFA-HT) media utilizing an aqueous matrix and stool samples from patients diagnosed with CDI over a range of bacterial densities.

ChromID CDIF agar was purchased from bioMérieux as commercially prepared plates. CCFA-HT plates were obtained from Anaerobe Systems (Morgan Hill, CA, USA) as commercially prepared, prereduced, individually packaged plates. All plates were removed from the packaging and transferred to an anaerobic jar within 30 minutes following sample plating.

To evaluate the growth of *C. difficile* on these selective media, *C. difficile* ATCC 9689 strain was transferred from a frozen stock onto anaerobic blood agar plates and grown anaerobically overnight at 37°C. A second subculture was performed and incubated similarly. From the second subculture, a 0.5–1 McFarland standard bacterial suspension was prepared in phosphate-buffered saline (PBS). Serial 10-fold dilutions were plated onto ChromID CDIF agar and CCFA-HT agar. This procedure was repeated seven independent times. The ChromID CDIF and CCFA-HT plates were incubated under anaerobic at 37°C conditions for 24 hours and 48 hours, respectively, before enumeration of colony counts.

Quantitation of *C. difficile* from patients with laboratory-confirmed CDI by PCR testing was then performed after obtaining informed consent. Fecal samples from patients diagnosed with CDI were obtained from the microbiology laboratory or directly from the patient prior to, during, or after receiving treatment. Within 12 hours of the bowel movement, the stool sample was obtained and processed. A sufficient amount of the stool sample (0.5–1 g) was weighed and an equal volume of PBS added. This primary emulsion was mixed thoroughly. To obtain spores, an aliquot (0.5–1 g) was taken from the primary emulsion, weighed, and diluted with an equal part of 95% EtOH (POST sample) and placed on an orbital shaker for 60 minutes. Another aliquot was taken from the primary emulsion, weighed, and diluted with an equal part of PBS (PRE sample). This sample included vegetative cells of *C. difficile* as well as spores. The POST and PRE samples were a final 1:4 dilution of the original patient fecal sample. Once the samples were diluted to a final 1:4 dilution, serial 10-fold dilutions of each sample were plated onto ChromID CDIF and CCFA-HT plates in duplicate. The plates were incubated as described above. Vegetative cells were calculated by taking the total colony-forming units (CFU)/g from the PRE plates and subtracting the total CFU/g from the POST plates. This process was repeated for 46 individual samples.

In the aqueous matrix, the bacterial density on ChromID CDIF read at 24 hours was similar to that on CCFA-HT read at 48 hours, $6.66 \pm 0.62 \log_{10}\text{CFU/mL}$ and $6.13 \pm 1.23 \log_{10}\text{CFU/mL}$ ($p = 0.124$), respectively. The bacterial density in fecal samples from patients with CDI on ChromID

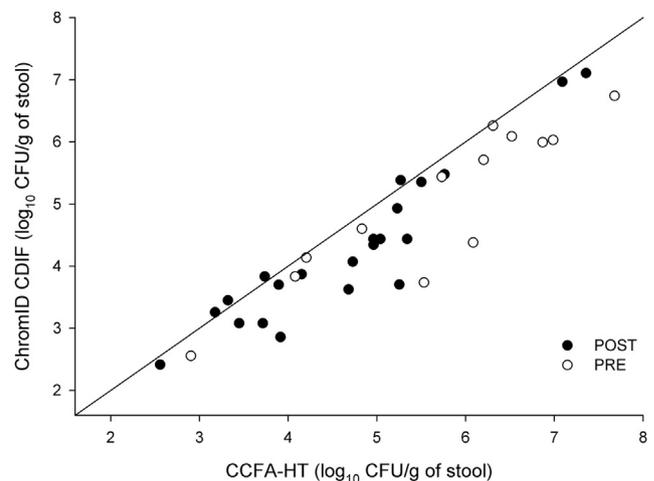


Figure 1. Comparison of *Clostridium difficile* densities in fecal samples from patients with *C. difficile* infections.

CDIF versus CCFA-HT is depicted in Figure 1. Recovery by quantitative methods was similar albeit slightly lower on ChromID CDIF compared with CCFA-HT media. *C. difficile* growth was present on 23/46 (50%) of samples for both CCFA-HT media and ChromID CDIF media following EtOH exposure (POST). From the PRE samples, *C. difficile* was cultured from 15/46 samples (33%) on CCFA-HT agar and 27/46 samples (59%) on ChromID CDIF media. Fourteen PRE samples had normal flora on the CCFA-HT media but not on the ChromID CDIF media, inhibiting enumeration and isolation of *C. difficile*. Three samples with the greatest differences were repeated in duplicate. Variability among individual samples was present but we observed similar results when enumeration was done an additional two times.

The use of chromogenic media to identify specific species in a clinical setting has provided relatively quick detection and quantitation of infecting pathogens. In the current study, we systematically studied the ability to quantitate *C. difficile* in a saline solution as well as isolate and quantitate the organism from stool utilizing the ChromID CDIF media compared with the gold standard, CCFA-HT media. In PBS, similar bacterial densities were present on the two types of media indicating that the media provides sufficient growth. Utilizing samples from multiple patients, ChromID CDIF media was able to isolate and quantitate *C. difficile* from more samples than with CCFA-HT. This was accomplished with a reduced incubation period and without the need for EtOH pretreatment, reducing both preparation time and turn-around time. This reduced turn-around time was also highlighted by Carson and colleagues.⁶ Further, the authors semiquantitatively assessed *C. difficile* growth in fecal samples utilizing isolates present in the first, second, third, and/or fourth quadrant. The current study was able to expand on the initial work of Carson et al.⁶ and perform a quantitative assessment utilizing absolute $\log_{10}\text{CFU/g}$ of stool concentrations and compare ChromID CDIF with CCFA-HT media.

It is important to note that when considering duplicate quantitative cultures for both media, variability in bacterial densities were observed. As the variability was noted on

both types of media, it is hypothesized this is due in part to the minimal diluting done to maintain sufficient bacterial counts above the lower limit of detection and the consistency of the sample. Stool samples ranged from Bristol stool scale 3–7 and we did not observe any correlation between variability and a lower or higher Bristol stool scale.

The ability to isolate and quantify *C. difficile* in fecal matter from patients using ChromID CDIF has important clinical, economic, and research implications. As microbiology laboratories and health systems continue to look for the most efficient use of new technologies, ChromID CDIF is able to reduce normal flora that the CCFA-HT media does not, allowing effective detection and quantitation of *C. difficile* in resource limited environments. The quantitation methods described herein provide a framework to conduct research to better understand the bacterial concentration of *C. difficile* within stool samples of patients with CDI.

Conflicts of interest

All authors have no conflicts of interest to note.

Acknowledgments

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