Comparison of Recovery Rates of *Clostridium difficile* from Stool Using Chromogenic Agar versus a Classic Culture Method

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Abstract

Background: The goal of this study was to compare performance characteristics of a conventional medium, taurocholate, cycloserine, cefoxitin, fructose agar (TCCFA) with CHROMagar™ *C.difficile* (CHROMagar Paris , France) for recovery of *C. difficile* from stool.

Methods: 50 stool specimens in Cary Blair that were known to be *C. difficile* PCR positive were subcultured in parallel to CHROMagar™ and TCCFA agars. Specimens were plated directly to both selective agars without processing prior to inoculation, and incubated in anaerobe jars with anaerobiosis attained using an AnaeroPack anaerobic gas generating system (Mitsubishi Gas Chemical America, Inc. New York, NY). The CHROMagar™ medium was examined at 24 hours in accordance with the manufacturer's recommendation, and tested within 6 hours of examination. Identification was performed by MALDI-TOF mass spectrometry directly from the CHROMagar™ plate. TCCFA medium was examined at 48 (±6) hours, in accordance with standard protocols. Identification was performed by MALDI-TOF mass spectrometry directly from selective plates, except in circumstances where subculture was required for isolation of colonies resembling *C. difficile*. Sensitivities of the two tests were compared using McNemar's test and detection duration was compared using a log rank test.

Results: CHROMagar™ media provided a 94% recovery rate of C. difficile on the first day of examination. Two specimens were positive on the first day, but were subcultured and identified on the second day. Three specimens did not yield *C. difficile* on CHROMagar™ media; *C. difficile* was recovered from one of the three specimens on TCCFA. Organisms other than *C. difficile* were not recovered on CHROMagar™. TCCFA agar provided a 68% recovery rate of *C. difficile*, requiring up to 4 days of incubation and occasional subculture to yield final results. Non-*C. difficile* bacteria were isolated on TCCFA. (Figure 1)

Conclusions: For culture-based screening of stool for *C. difficile*, the CHROMagar™ medium was more sensitive than the TCCFA medium (p=0.0008), with faster detection (p<0.0001).

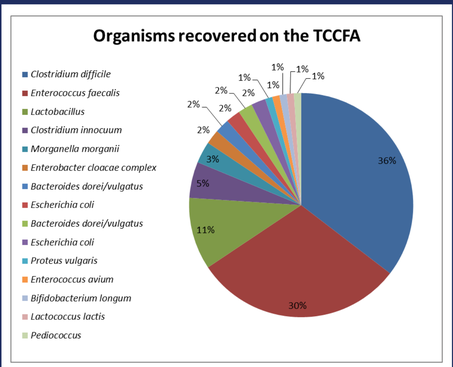
Objectives

Classical culture methods for recovery of *Clostridium difficile* from stool calls for alcohol treatment of the sample followed by plating on selective media (taurocholate, cycloserine, cefoxitin, fructose agar- TCCFA) with the initial examination at 48 hours.

The manufacturer suggests that CHROMagar™ *C.difficile* media will isolate *C. difficile* from untreated stool in 24 hours, dramatically reducing manipulation of the specimen and overall technologist time spent on recovery and identification.

The intent was to verify manufacturer's performance characteristics for selectivity and specificity (predominant or pure culture of *C. difficile* when present), sensitivity (ability to recover *C. difficile*) and speed of recovery (24 hours vs. 48 hours).

Figure 1



Methods

50 stool samples in Cary Blair that were known to be *C. difficile* positive based on PCR testing, were subcultured in parallel to CHROMagar™ and TCCFA agar for comparative recovery purposes. Samples were plated directly to both selective agars without processing prior to inoculation, and incubated in Anaerobe jars with anaerobiosis attained by AnaeroPack anaerobic gas generating system.

The CHROMagar™ medium was examined at 24 hours per the manufacturer's protocol and tested within 6 hours of examination. Definitive identification of colonies was performed by MALDI-TOF mass spectrometry directly from the CHROMagar plate.

The TCCFA agar was examined at 48 (±6) hours. Subculture to anaerobic sheep blood media was performed as necessary to isolate appropriate morphologies from the selective plates. Colony identification was performed by MALDI-TOF mass spectrometry directly from the selective plates, except in circumstances where subculture was required for colony isolation.

Comparative statistical analysis of the sensitivity of the two media was performed using McNemar's test and detection duration was calculated using a log rank test.

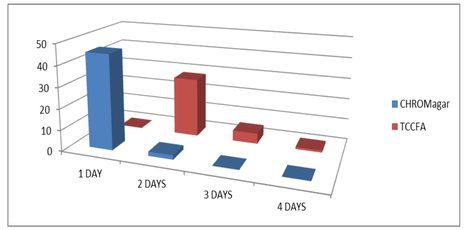
Discussion

The implementation of this culture methodology will allow for rapid and sensitive isolation of *C. difficile* from stool. Within the clinical setting, this represents an increased ability for the laboratory to recover isolates to provide susceptibility testing for patients.

This plate method also has the advantage of reducing the overall hands-on time traditionally required to isolate *C. difficile*.

These time-savings begin with specimen processing by eliminating the need to alcohol-treat the specimen, continue with reduced required incubation time, and conclude with the plates' greater ability to inhibit other intestinal flora such as *Enterococcus* and *Lactobacillus* species, thus improving confidence and reducing time spent manipulating the culture.

Figure 2: Comparative Recovery Times



Results

CHROMagar™ media provided a **94% recovery rate** of *C. difficile* on the first day of examination. Two specimens were positive on the first day, but were subcultured and identified on the second day. Three specimens did not yield *C. difficile* on CHROMagar™ media; *C. difficile* was recovered from one of these three specimens on TCCFA.

Organisms other than *C. difficile* were not recovered on CHROMagar™.

TCCFA agar provided a **68% recovery rate** of *C. difficile*, requiring up to 4 days of incubation and occasional subculture to recover.

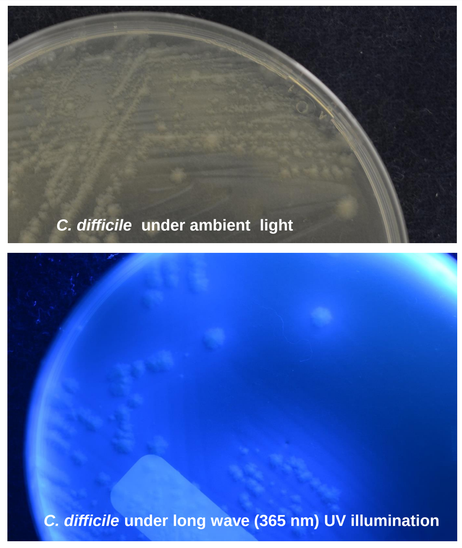
Several non-*C. difficile* organisms were isolated on TCCFA, which contributed to the need for subculture (Figure 1).

Overall, CHROMagar™ medium was more sensitive than TCCFA medium (p=0.0008), with faster detection (p<0.0001).

Conclusion

For culture-based testing of stool for *C. difficile*, CHROMagar™ medium was more sensitive than TCCFA medium and provided more rapid isolation.

Figure 3: *C. difficile* on CHROMagar



References

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