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

Methods: 50 stool samples in Cary Blair that were known to be C. difficile/PCR positive were subcultured in parallel to CHROMagar® C. difficile (CHROMagar Paris, France) and TCCFA agar. Specimens were plated directly to both selective agars without processing prior to inoculation, and incubated in anaerobic jars with anaerobiosis attained using AnaerPack 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 using MALDI-TOF mass spectrometry directly from the CHROMagar® plate. TCCFA medium was examined at 48 (±6) hours, in accordance with standard protocol. Identification was performed by MALDI-TOF mass spectrometry directly from the selective plates, except in circumstances where subculture was required for isolation of colonies resembling C. difficile. Sensitivities of the 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® or TCCFA agar despite a 98% recovery rate of C. difficile requiring up to 4 days of incubation and subculture. Non-C. difficile bacteria were isolated on TCCFA. (Figure 1)

Conclusions: For culture-based, stool testing for C. difficile, the CHROMagar® medium was more sensitive than the TCCFA medium (p<0.0006), with faster detection (p<0.0001).

Abstract

Classical culture methods for recovery of Clostridium difficile from stool calls for alcohol treatment of the specimen followed by plating on selective media (taurocholate, cycloserine, 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 technologic time spent on recovery and identification. The intent was to verify manufacturer’s performance characteristics for selectivity and specificity (purification or pure culture of C. difficile when present), sensitivity (ability to recover C. difficile) and speed of recovery (24 vs. 48 hours).

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 anaerobic jars with anaerobiosis attained using AnaerPack anaerobic gas generating system (Mitsubishi Gas Chemical America, Inc, New York, NY). 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.

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 to the laboratory to recover isolates to provide susceptibility testing for patients. These time-savings begin with specimen processing by eliminating the need to alcohol-treat the specimen, continue with reduced required incubation time and confirm colonies in 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.

Conclusion

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