Effects of Storage and Transport Conditions on the Viability of C. difficile in Soft or Liquid Human Stool Specimens

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REVISED ABSTRACT

Background: Culture methods for C. difficile lack standardization and there is a paucity of information on C. difficile viability during specimen handling. To better understand C. difficile recovery from stool specimens, we evaluated the use of Anaerobic Transport Medium (ATM, w/o ATM), use of an enrichment broth (Direct, Enriched), and effect of specimen shipping and storage time (7 days). Methods: The microbiology laboratories from the Johns Hopkins Hospital (JHH) and Wishart Health Services (WHS) cultured 30 stool specimens that were positive for the toxins by the BD GeneOhmTM Cdiff Assay (BD Diagnostics, Quebec, Canada). Positive specimens were performed (5-40h after collection) from JHH were aliquoted, stored in tubes with and w/o ATM (Anaerobe Systems, Morgan Hill, CA), and shipped via 48 to 72 h to JHH or WHS. Specimens were cultured anaerobically by JHH and WHS at days 3, 5 and 7 after collection using both direct plating to Cytosine Cefoxitin and Fructose Agar plate with Horse Blood (Remel, Lenexa, KS) and an enriched culture method using 75 ul of stool into Cytosine Cefoxitin Mannitol Broth with Taurocholate and Lysozyme (Anaerobe Systems, Morgan Hill, CA). Results: To assess repeated measures under different experimental conditions and across different time points, a logistic model with correlated data (SAS GENMOD procedure) was performed to determine if any of the four factors (Site, Transport Media, Culture Media or Time) had an effect on recovery of C. difficile. No difference was seen in the recovery of C. difficile when assessing the shipment of the samples (JHH vs WHS; univariate analysis, p=0.3021), in recovery from samples with or w/o ATM for any day of testing using direct or enriched culture (multivariate analysis, p=0.027). At both sites, the viability of C. difficile was similar at all storage time points, direct culture recovery was 81.9 to 97.3% (p=0.028) and enriched culture recovery was 97.4% (95% CI: 97.0-97.8) (univariate analysis, p=0.088). However, more isolates were recovered using an enrichment broth (multivariate analysis, p=0.0072). Conclusion: The shipment of stool specimens does not have an impact on the recovery of C. difficile and the use of ATM does not increase the recovery of C. difficile from stool specimens stored up to 7 days at 2-8 °C. Better recovery of C. difficile was observed using an enriched culture method.

INTRODUCTION

Since the mid-1970’s, toxigenic C. difficile has been recognized as the cause of pseudomembranous colitis and antibiotic associated diarrhea. The prevalence and virulence of the disease has substantially increased over the last decade. The epidemic of C. difficile disease has fostered development of a variety of molecular methods more sensitive than toxin detection by enzyme immunoassays. Toxinogenic anaerobic culture has re-emerged as a standard against which these assays are compared, although there is no agreed upon standard and few if any papers examine viability of the organism over time prior to culture.

Most research examining the viability and recovery of C. difficile in culture has involved the use of innovative selective and differentiation agents for its isolation, attempts to determine the most appropriate enrichment broth method, or elucidation of the number of stool specimens necessary to guarantee recovery. Non-molecular methods involving the use of different media for the detection of C. difficile in the stool may have the most appropriate cytotoxic assay to determine toxicity or to elicit cytoxicity stability over time.

GOALS OF THE STUDY

- We assessed the viability of C. difficile in liquid and soft stool specimens in order to establish the transport and storage time limit, and optimal conditions in which a stool specimen should be held before culture isolation. Stool specimens testing positive for C. difficile using the BD GeneOhm™ Cdiff Assay (Cdiff) (BD Diagnostics, Quebec, Canada) were cultured without and with the use of Anaerobic Transport Medium (ATM) (Anaerobe Systems, Morgan Hill, CA) and with the use of pre-reduced CCA-HB alone (Direct) or with the use of an enrichment broth Cytosine Cefoxitin Mannitol Broth with Taurocholate and Lysozyme (CCMB-9LX) (Enriched). Additionally, the culture method was performed in parallel at the Johns Hopkins Hospital (JHH) and Wishart Health Services (WHS) using three time points (day 3, day 5, and day 7) for each sample to determine the impact of transportation and/or storage on C. difficile recovery.
- Four variables were analyzed to determine their effects on the recovery of Clostridium difficile from human stool samples: Specimen shipping and site (between JHH and WHS), Effect of Anaerobic Transport Medium (ATM vs. w/o ATM), Storage time at 3, day 5, and day 7.

- This study was approved by the Johns Hopkins University School of Medicine Institutional Review Board and Indiana University Office of Research Administration.

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RESULTS

Specimen Selection: Stool samples were obtained from 125 ml liquid stool or soft stool specimens (~12 ml, 5-40 hrs post-collection) submitted to the JHH laboratory that tested positive for the toxin by the BD GeneOhmTM Cdiff Assay (BD Diagnostics, Quebec, Canada) and were cultured positive within 48 hrs of collection and divided into aliquots (1 ml each).

Specimen Storage and Shipping Conditions: All aliquots were stored at 4 °C at JHH, with and without ATM. Shipped to WHS <72hrs after collection at 2-6 °C, with and without ATM.

Culture Setup: Anaerobic culture was performed at both sites on days 3, 5, and 7 post-collection using an aliquot stored at 4 °C with and without ATM.

Enrichment Broth: Anaerobic cultures were directly inoculated onto a pre-reduced CCA-HB plate (Remel, Lenexa, KS) (Direct Culture) and <75 ul of stool into CCMB-9LX (Anaerobe Systems, Morgan Hill, CA) (Enriched Culture).

Cultures were incubated at 37 °C in an anaerobic chamber and read at 48 hrs. If cultures were negative at 48 hrs, they were incubated an additional 72 hrs before being called negative.

Enriched cultures were exposed to aerobic conditions only when ready to identify or upon subculturing.

Identification of colonies morphologically compatible with C. difficile was confirmed as follows:
- Gram positive bacilli
- Characteristic barnyard-like odor
- Aerocultured on chocolate agar plate incubated 48 hrs at 37 °C in 5% CO2
- Pro-disk (L-proline-β-naphthylamide) test positive [Key Scientific Product, Inc. Stamford, CT]

Effect of Anaerobic Transport Media (ATM vs. w/o ATM).

Between JHH and WHS: no difference was observed in the univariate and multivariate analyses when comparing recovery of C. difficile by either culture method.

Use of ATM: no difference was observed with or without the use of anaerobic transport media by univariate and multivariate analyses.

Direct culture and time: Recovery of C. difficile decreased between day 3 and 7 but there was no statistical difference by univariate and multivariate analyses.

Enriched culture and time: Recovery of C. difficile remained the same between day 3 and 7 and there was no statistical difference by univariate and multivariate analyses.

Direct culture and enriched culture: Statistical difference was observed at day 7 with the univariate analysis and the difference was also observed in the multivariate analysis (p=0.0071).

Recruitment of C. difficile was not affected by shipping and culture location; use of the ATM did not affect recovery of C. difficile and, storage of the specimen for 7 days did not affect the recovery of C. difficile.

The use of a CCMB-9LX improved the recovery of C. difficile from stool in anaerobic culture.

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