Detection of Clostridium difficile Toxin in Patient Samples by Cell Culture Cytotoxicity Neutralization Assay (CCNA) Using Isolated Colonies of C. difficile Versus Stool Samples

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Abstract

BACKGROUND: Clostridium difficile toxin is a common cause of antibiotic associated diarrhea (AAD) and pseudomembranous colitis (PMC). The toxigenic culture method in which organisms are cultured and the stool is tested by the Cell Culture Cytotoxicity Neutralization assay (CCNA) remains the diagnostic ‘gold standard’ for detection of toxigenic C. difficile. The CCNA consists of two reactions—detection of toxin and toxin neutralization by the C. difficile antitoxin. The method is extremely sensitive and can detect toxin levels as low as 1 picogram. However, our evaluation shows that when a pure isolate of C. difficile is used as an alternative specimen, the sensitivity of the test is higher than the raw stool specimen.

METHOD: Stool samples tested—preserved samples in Cary-Blair transport medium (stored ambient) and unpreserved samples (stored at -70°C). Preserved stool from Cary-Blair medium was cultured anaerobically for growth of C. difficile. 27 stool cultures that yielded C. difficile were tested. Isolated colonies for these 27 samples along with C. difficile strains ATCC9689 (toxin producing) and ATCC700057 (non-toxin producing) were tested. 2 to 3 colonies of C. difficile isolate were inoculated into cooked meat broth and thioglycolate broth. CCNA using the Diagnostic Hybrids Cytotoxicity Assay was performed on both broth types after 48 hours of incubation (anaerobically). For the preserved samples that were positive by culture, the corresponding preserved samples leading to a false negative result.

RESULTS: 9 out of the 27 unpreserved stool samples were cytotoxin-positive, and 18 were negative on direct testing. Of the 27 C. difficile isolates, 22 confirmed cytotoxin production with both broths; 1 tested positive and 18 were negative on direct testing. Of the 27 cultures, the sensitivity of the test is higher than the raw stool sample was also tested for CCNA.

CONCLUSIONS: A delay in transport of unpreserved stool samples can lead to degradation of toxin in samples leading to a false negative result. CCNA testing of C. difficile isolates from preserved stool samples (enriched in cooked meat broth or thioglycolate broth) provides a higher level of sensitivity than that obtained with direct testing of unpreserved stool samples.

Introduction

- Clostridium difficile toxin is a common cause of antibiotic associated diarrhea (AAD) and pseudomembranous colitis (PMC).
- The current “gold standard” for detection of toxigenic C. difficile is toxigenic culture followed by the Cell Culture Cytotoxicity Neutralization assay (CCNA).
- The CCNA method is extremely sensitive and can detect toxin levels as low as 1 picogram.
- The Diagnostic Hybrids Cytotoxicity Assay for the CCNA method should be performed using stool specimen filtrate.
- Sensitivity of this method can be increased further by utilizing C. difficile isolates as alternative specimen.

Materials & Method

- C. difficile isolates recovered from stool samples cultured from a Para-Pak® C&S container (Meridian Biosciences, Inc.), which is a modified Cary-Blair medium
- Diagnostic Hybrids Cytotoxicity Assay for C. difficile toxin by tissue culture method was performed to determine the presence of toxin
- C. difficile colonies on CCFA
- C. difficile cell culture plate containing MRHF cells
- C. difficile toxin positive
- C. difficile toxin negative
- Antitoxin reagent
- Toxic control
- Specimen diluent

Qualitative Method Comparison

Ref. Method: Stool Test Method: Isolate

Experimental Results

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Results

- Experimental Results
- Statistical Analysis

Conclusions

- C. difficile infection is one of the major causative agents of nosocomial intestinal infections—especially in adults.
- The symptoms of C. difficile infection are essentially caused by the production of toxins.
- A delay in transport of unpreserved stool samples can lead to degradation of toxin in samples leading to a false negative result.
- Detection of toxin directly from stool varies and is mostly dependent on the specimen quality and its subsequent handling.
- Detection of toxin from C. difficile colonies isolated from preserved stool samples (enriched in cooked meat broth or thioglycolate broth) results in a higher level of sensitivity as well as a reduction in the number of false negative results.
- In cases where time and technical skills are not an issue, this is the optimum method for toxin detection.

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