**Clostridium difficile** outbreaks

To the Editor:
The seriousness of **Clostridium difficile** outbreaks in Montreal, Quebec, and other areas of Quebec has been well described in the recent literature (1,2). Currently, a hypervirulent strain of **C difficile** is making its presence felt, causing morbidity and mortality related to **C difficile-associated disease** (CDAD).

The laboratory detection of **C difficile** and its toxins is essential for the diagnosis of CDAD; the available methods have been described (2). Controversy exists about which of the several methods for the detection of **C difficile** and its toxins is optimal. Enzyme immunosassays are the tests most commonly used. Toxin A or toxins A and B can be detected; however the sensitivity of 65% to 85% is too low. Cytotoxin B assay is the ‘gold standard’, with a sensitivity of 80% to 90% and an excellent specificity of 99% to 100% (2). The test results are not available until after 24 h to 48 h, which is one of the principal limitations of the test, according to the literature.

The Society for Healthcare Epidemiology of America recommends both cytotoxin assay and culture for maximal diagnostic sensitivity and specificity (3). Several authors have followed this optimal strategy, and 15% to 43.6% of additional toxin-producing strains have been detected (4). The charts of patients with positive toxigenic culture were analyzed and they indeed had CDAD. This method requires technical expertise that is not available in all laboratories.

Recently, marketed enzyme immunosassays for glutamate dehydrogenase (GDH) were evaluated, and the results were compared with those obtained by **C difficile** culture. Sensitivity was 84.9% to 93.5% with a negative predictive value of 98.5% to 99.6% (5). However, GDH tests, such as stool cultures for **C difficile**, were unable to differentiate between toxigenic and nontoxigenic strains. The sensitivity of the enzyme immunosassay approach for detecting toxins A and B has been found to be unacceptably low (38%) by others (6).

Detection of toxigenic **C difficile** by a two-step algorithm, including GDH antigen testing (C. DIFF CHEK-60, TechLab Inc/Wampole Inc, USA) and cytotoxin assay for antigen-positive specimens has been evaluated. Antigen-negative results were 99.7% predictive of cytotoxin assay negativity. Antigen enzyme immunosassay sensitivity, specificity and positive predictive value were 98%, 89.3% and 53%, respectively (6). When the authors used this approach in their hospital, physicians obtained useful information within one day of specimen receipt. Antigen detection is reported, and if positive, practitioners know that cytotoxin assay is pending. The authors incubate the cytotoxin assay for as long as 48 h, but 75% to 80% of cytotoxin-positive specimens are identified after 24 h. This approach is also less expensive than using cytotoxin assay alone (6). The GDH method also allows for increased sensitivity of the cytotoxin assay with **C difficile** culture, followed by testing isolates for toxin production in the cases in which GDH assay is positive and the cytotoxin assay is negative.

The current Quebec epidemic strain has been characterised by pulsed-field gel electrophoresis (PFGE) and polymerase chain reaction ribotyping as North American PFGE type 1 and polymerase chain reaction ribotype 027 (7). Concentrations of toxins A and B in this strain are 16 and 23 times higher, respectively, than other PFGE types (7). With such a high concentration of toxin B, we hypothesized that a cytopathic effect could be detected early in most specimens tested. An earlier study has described a cytopathic effect after 4 h with **C difficile** crude toxin and with specimens from patients with pseudomembranous colitis and a high titre of toxin (8). However, this study did not address CDAD presenting other than as pseudomembranous colitis, and recent literature does not report the optimal time to examine cell cultures.

We evaluated the detection of the cytopathic effect of stool filtrates after 4 h using the cell culture technique used in our hospital. Fresh stools were diluted in phosphate buffer saline (pH 7.5), vortexed and centrifuged. The supernatant was filtered and final dilutions (1:10 and 1:100) of stool filtrate were inoculated in a 96-well microtitre plate containing the MRC-5 fibroblast cell line. One specimen that diluted at 1:10 was also inoculated with **C difficile** antitoxin. The cells were examined at 24 h, 48 h and 72 h for cytopathic effects. The stool filtrates were kept at 4°C, and the specimens that were positive after 24 h were retested the following day, or no later than 72 h. Of 385 fresh specimens tested, 52 (13.5%) were positive for the **C difficile** toxigen. Of these 52 specimens, 37 (71.2%) were positive after 24 h. These 37 specimens were retested and 24 (64.9%) were found positive within 4 h.

This approach allowed the detection of 24 of 52 (46.1%) positive specimens at 4 h. This early detection offers a great advantage for the care of infected patients. The cytotoxin assay may be used alone or in combination with GDH assay, and a reading made after 4 h gives definitive results in nearly one-half of patients with CDAD.

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