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Evaluation of a Commercial Kit for the Routine Detection of *Clostridium difficile* Cytotoxin by Tissue Culture

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The Toxi-titer microtiter plate system (Bartels Immunodiagnostic Supplies, Inc., Bellevue, Wash.) is a simplified procedure for detecting the cytotoxin produced by *Clostridium difficile* in stool filtrates. In a parallel study of 74 stool specimens, results from the Toxin-titer system compared favorably with those from the conventional system. Our experience with the Toxin-titer system in testing 540 stool specimens was, in general, satisfactory, although a few problems with toxin control did occur. The method requires no specialized experience in tissue culture techniques and can therefore be adopted for routine use in clinical laboratories.

The increased awareness of the role of *Clostridium difficile* toxin(s) in antibiotic-associated diarrhea has resulted in more frequent requests by clinical laboratories for the detection of the toxin(s) in fecal specimens. The test most commonly used to diagnose antibiotic-associated diarrhea is the detection of the cytotoxicity of the toxin in fecal specimens by a tissue culture assay and neutralization of the toxin by *C. difficile* or *C. sordellii*-antiserum. However, many clinical laboratories are not equipped for tissue culture assays. Alternatives to the tissue culture assay, such as counterimmunoelectrophoresis, enzyme-linked immunosorbent assay, and latex agglutination, have been proposed as immunodiagnostic tests for antibiotic-associated diarrhea. When available antisera produced by a *C. difficile* culture filtrate were used, counterimmunoelectrophoresis was found to have a low sensitivity and specificity as compared with the tissue culture assay (2). Laughon et al. (1) developed separate enzyme-linked immunosorbent assays for *C. difficile* cytotoxin and enterotoxin with antisera produced by purified cytotoxin and enterotoxin and reported sensitivities of 80 and 90% and specificities of 100 and 98.6%, respectively. Although the sensitivities and specificities of the enzyme-linked immunosorbent assays were good, the procedure is too laborious to be useful in most clinical laboratories. More recently, Ryan et al. (R. W. Ryan, I. Kwasnik, and R. C. Tilton, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 163, 1985) evaluated a rapid latex agglutination test (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.) for the detection of enterotoxin in fecal specimens and reported a sensitivity of 88% and a specificity of 98.8% as compared with the tissue culture assay. Latex agglutination detects enterotoxin, and the tissue culture assay detects cytotoxin. Whether latex agglutination can be used as an alternative or adjunct test to the tissue culture assay for the diagnosis of antibiotic-associated diarrhea remains to be determined.

In this paper we report the results of a cytotoxicity assay with a commercial kit, Toxi-titer (Bartels Immunodiagnostic Supplies, Inc., Bellevue, Wash.), and compare this system to a conventional tissue culture system. The Toxi-titer microtiter plate contains six rows of eight microtiter wells, each seeded with 75 to 100% confluent growth of viable human foreskin fibroblast cells, ready for the cytotoxicity assay without additional processing. Each row is covered with a sterile plastic strip designed to prevent the medium from drying. Other materials included in the kit are a toxin control, an antiserum prepared against *C. difficile* toxin, and a diluent for preparing the stool filtrate.

In both assay systems, stool filtrates at final concentrations of 1:40 and 1:80 were used. Procedures outlined by the manufacturer were followed for the Toxi-titer assay. Procedures for the conventional assay were described previously (2). The cell line used in the present study was WI-38 human fibroblast (Flow Laboratories, Inc., McLean, Va.). Both *C. difficile* toxin and antitoxin antiserum were purchased from Virginia Polytechnic Institute, Blacksburg, Va. In both systems, a positive assay was defined as a minimum of 25% of cell rounding that could be neutralized by the antitoxin. Nonspecific cytopathic effects caused by viral agents in the stool filtrates were defined as nonneutralizable rounding of the cell monolayer. Nonspecific toxic effects caused by toxic substances in the stool filtrates were defined as granularity, rounding, and sloughing of the cell monolayer with or without the addition of antitoxin. To compare the sensitivity of these two systems, we tested cytotoxin-positive specimens at dilutions of 1:40 to 1:3,200,000. A total of 74 stool filtrates were tested in parallel by both systems. Eleven cytotoxin-positive specimens were detected by both systems. Of these three had the same titers in both systems two had 2-fold higher titers in the conventional system, and six had 10- or 100-fold higher titers in the Toxi-titer system. Of 63 cytotoxin-negative specimens, 9 exhibited nonspecific toxic effects at a dilution of 1:40 but not at a dilution of 1:80 after 24 h of incubation in the Toxi-titer system. No nonspecific toxic effects were detected in the conventional system.

We have since used the Toxi-titer system for the detection of *C. difficile* cytotoxin in 540 stool specimens. Of 29 cytotoxin-positive specimens, 21 (72%) were positive at 24 h, and 8 (28%) were positive at 48 h. In all positive specimens, both 1:40 and 1:80 dilutions had a minimum of 50% cell rounding. Nonspecific toxic effects occurred at a rate of 5%. One specimen exhibited nonspecific cytopathic effects caused by a possible viral agent present in the stool. The conditions of the microtiter plates upon receipt were generally satisfactory. Only 1 of 69 plates was found to be contaminated on arrival during this study period. The toxin control always exhibited 100% cytopathic effects throughout the cell culture monolayer at a working dilution of 1:2.

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However, three lots of the toxin control exhibited less than 25% cytopathic effects at a 1:2 dilution, and two lots had to be diluted to 1:4 to be neutralized completely. In both situations, the quality controls of the cell culture and the antitoxin were found to be satisfactory.

In general, we have been pleased with the performance of the Toxi-titer system. We recommend the routine use of two dilutions of the same specimen for the cytotoxicity assay. The advantages are the reinforcement of a positive result and the minimization of the problem of nonspecific toxic effects at lower dilutions. The Toxi-titer system is versatile, since columns of microtiter wells can be used on different days or several columns can be used on the same day if multiple specimens are to be tested. The cell culture has a shelf life of 2 weeks without the need of changing the medium. We conclude that the Toxi-titer system is simple, economical, and amenable to being adopted for routine use in clinical laboratories without tissue culture experience.

LITERATURE CITED

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