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Evaluation of a Commercial Cytotoxicity Assay for Detection of *Clostridium difficile* Toxin

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A comparative study was performed to determine the accuracy of *Clostridium difficile* toxin detection. A commercial cytotoxicity assay (Bartels Immunodiagnostic Supply, Bellevue, Wash.) was compared with conventional microcytotoxicity assays, using Vero and MRC-5 cells. The Bartels system was found to be essentially equivalent to conventional cytotoxicity assays currently being performed for routine *C. difficile* toxin detection.

Clostridium difficile is a major cause of antibiotic-associated diarrhea and colitis (2). The disease is mediated by the production of an enterotoxin by the bacterium; however, the laboratory diagnosis is based on the presence of a cytotoxin, easily detected by cell culture techniques. Until recently, only laboratories with the capability of performing cell culture techniques could test for *Clostridium difficile* toxin. Recently, a complete commercial system for detecting this toxin was introduced for laboratory testing by Bartels Immunodiagnostic Supplies, Inc., Bellevue, Wash. The Bartels system is similar to other conventional methods in that the detection of *C. difficile* toxin is determined by the effect of the toxin, present in stool samples, on tissue culture cells and confirmed by toxin neutralization. Because this system allows laboratories not equipped for tissue culture assays to test routinely for *C. difficile* toxin, we evaluated the kit and compared it with a conventional cytotoxicity assay, using two different cell lines.

Stool filtrates for the Bartels and MRC-5 assays were prepared in the following manner. A portion of fresh stool specimen or a sample frozen at −70°C was placed in a microcentrifuge tube and mixed with an equivalent amount of phosphate-buffered saline. The specimen was centrifuged at 10,000 × *g* (with the Fisher microcentrifuge 235A; Fisher Scientific Co., Pittsburgh, Pa.) for 10 minutes to pellet the solid material. The supernatant was transferred to a sterile test tube and frozen at −70°C. When ready for testing, the supernatant was thawed at room temperature and passed through a 0.45-μm-pore filter (Acrodisc; Gelman Sciences, Inc., Ann Arbor, Mich.) and the filtrate was collected in a sterile tube. An equivalent amount of sterile saline was then added and mixed. This was the working filtrate used for the Bartels and MRC-5 assays.

The reference method was performed with microtiter plates, as specified by Gilligan et al. (4), using Vero cells for toxin detection. A modification of the above procedure with MRC-5 cells was also tried. For both cytotoxicity assays, the toxin (1) and antitoxin (3) were purchased from T. D. Wilkins, Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg. The assays were performed essentially as described in the accompanying package insert.

The Bartels cytotoxicity assay was supplied as a complete kit and contained one Toxi-titer microtiter plate with human foreskin fibroblasts, rabbit anti-*C. difficile* toxin, positive toxin control, and diluent. The microtiter plate contained six columns of microtiter wells with cells. Each column contained a sterile plastic strip designed to maintain proper pH and prevent drying.

The procedure for the assay was essentially the same as for the reference method. Dilutions of stool filtrate were preincubated with diluent, and antitoxin was placed on the tissue culture cells. If the stool contained toxin, cells appeared round with cytoplasmic projections, an effect that was neutralized in the presence of the specific antitoxin. A test was considered positive when any dilution of filtrate showed neutralization. The plates were incubated for 24 to 48 h at 35 to 37°C (CO_2 was not required).

In phase 1, 211 samples were tested by the Bartels and the reference (Vero cells) methods. A total of 77 samples were toxin positive by the reference method, and 74 were positive by the Bartels method (96.1% sensitivity). Of the 132 samples that were negative by the reference method, 131 were negative by the Bartels method (99.2% specificity). The one discrepancy showed nonspecific reactivity (toxicity in the antitoxin well) by the Bartels method. If the discrepancy were excluded, specificity would reach 100%. Of the samples showing toxin-positive results, 92% showed positive results after 24 h of incubation, and the remainder became positive within 48 h of incubation.

In phase 2, 127 samples were tested with the Bartels method, the Vero cell method, and a cytotoxicity assay with MRC-5 cells. Of 12 samples found to be toxin positive by the Vero cell method, 11 were found positive by both Bartels and MRC-5 (91.6% sensitivity). The one false-negative result was shown by the reference procedure to have a low toxin titer but was not detected with the standard filtrate dilutions of the commercial system. The specificity of the Bartels and MRC-5 methods was 100% with no nonspecific reactions detected in this phase of the study.

The combined results of the Bartels and Vero cell methods are summarized in Table 1. When data from both phase 1 and 2 were combined and nonspecific reactions were excluded from the analysis, the Bartels system showed 94.4% sensitivity and 100% specificity.

The results of this study showed that the Bartels system performed very well when compared with the conventional cell culture methods for detection of *C. difficile* toxin. The Bartels system was easy to use, came complete with the necessary reagents to perform the cytotoxicity assay, and

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TABLE 1. Two-phase comparison[a] of the Bartels cytotoxicity assay with the Vero cell method

Bartels reaction	No. of tests by Vero cell reaction:	
	Positive	Negative
Positive	84	0
Negative	5	246

[a] Nonspecific reactions excluded.

did not require expertise in preparing cell cultures. In addition, a CO_2 incubator was not required, since the wells were sealed during storage and incubation. Also, a bright-field microscope could be used to read the results, although an inverted phase-contrast microscope would be preferred.

The ability to test for *C. difficile* toxin in laboratories not equipped for cell culture is desirable. It appears that the Bartels bioassay is an acceptable substitute for standard cell culture assays. Further development of rapid immunoassays such as the enzyme-linked immunosorbent assay (6) and latex agglutination (5, 7) would be the most desirable for those laboratories interested in alternative testing.

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