

## NOTES

### Detection of *Clostridium difficile* Toxin in Various Tissue Culture Monolayers

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**Thirty stool filtrates known to contain *Clostridium difficile* toxin based on previous testing on McCoy cells were tested for toxicity on primary African green monkey kidney (AGMK), McCoy, MRC-5, primary rhesus monkey kidney (RMK), and Vero cells. All 30 filtrates showed cytotoxic effect at  $\geq 1:100$  dilution on McCoy and Vero cells. A total of 22 filtrates were positive on MRC-5 monolayers, while only 16 and 10 filtrates showed positive cytotoxic effect on AGMK and RMK cells, respectively. Another 630 stool specimens were tested on McCoy and Vero cells only. Of these stool filtrates, 70 were positive and 560 were negative with both cell lines, which thus gave 100% agreement. Vero cells can be used interchangeably with McCoy cells for the detection of *C. difficile* toxin in stool filtrates.**

Detection of *Clostridium difficile* cytotoxin in stools from patients with diarrhea or pseudomembranous colitis must be rapid for optimal patient care. Testing for cell culture cytotoxicity is an accepted procedure (2, 3, 5, 6) for the detection of *C. difficile* cytotoxin within 24 h of inoculation. However, delays in testing may result if the specimen arrives when no suitable cell culture is available.

This study was undertaken to determine which cell lines available in our laboratory could be used to detect *C. difficile* toxin in stool filtrates. Since HeLa and McCoy cell monolayers were previously shown to have no significant difference in the detection of *C. difficile* toxin (6), HeLa cells were not included in this study. Vero cells (used in our laboratory for the detection of Vero toxin from *Escherichia coli* O157:H9) and cell lines used in our virus laboratory were included in the study. Thus, the five cell lines used for the initial determination of toxin detection sensitivity were primary African green monkey kidney (AGMK), McCoy, MRC-5, primary rhesus monkey kidney (RMK), and Vero cells.

Cell cultures were grown in polystyrene 150-cm<sup>2</sup> tissue culture flasks (Corning Glass Works, Corning, N.Y.) with Eagle minimal essential medium (Autopow; Flow Laboratories, Mississauga, Ontario, Canada), to which were added 2 mM L-glutamine, 5  $\mu$ g of gentamicin per ml, 25 U of mycostatin per ml, sufficient volume of 7.5% sodium bicarbonate to bring the solution to pH 7.2, and 10% heat-inactivated fetal bovine serum (GIBCO Laboratories, Burlington, Ontario, Canada). The cells were trypsinized by using 0.25% trypsin with 0.02% EDTA in phosphate-buffered saline (pH 7.2; PBS). A viable cell count was performed with erythrosine B (0.08% in PBS), and preparations showing  $\geq 90\%$  viable cells were used. The cells were then diluted to  $1.5 \times 10^5$  viable cells in growth medium for seeding.

Thirty stools previously tested on McCoy cells and known to contain *C. difficile* toxin (stored at  $-70^\circ\text{C}$ ) were used for the detection of cytotoxin by the five cell lines mentioned above. Stool filtrates were prepared by emulsifying speci-

mens in a 1:10 dilution with PBS and centrifuged for 10 min at  $2,500 \times g$ . Supernatants were filtered through membranes (pore size, 0.45  $\mu$ m; Millipore Ltd., Mississauga, Ontario, Canada) and either tested on the same day or kept at  $-70^\circ\text{C}$  if delay was anticipated. Filtrates were further diluted to 1:20, 1:50, 1:100, 1:200, 1:400, and 1:800 in PBS. These stool filtrates were added to tissue cultures grown for 24 h in flat-bottom 96-well microtiter plates (Costar, Cambridge, Mass.). A 20- $\mu$ l sample of each diluted filtrate, including the initial filtrates, was added to four wells, and two of the wells received 20  $\mu$ l of a 1:50 PBS dilution of *Clostridium sordellii* antitoxin (Wellcome Research Laboratories, Beckenham, United Kingdom) to neutralize the toxin. Since each well contained 0.2 ml of tissue culture fluid, 20  $\mu$ l further diluted every filtrate by 1:10. The final filtrate dilutions in the tissue culture wells thus were 1:100, 1:200, 1:500, 1:1,000, 1:2,000, 1:4,000, and 1:8,000. To obtain a 1:50 dilution, 40  $\mu$ l of the initial stool filtrate was added to the four wells in each test (see Table 1). Filtrates were tested in duplicate. Positive, negative, and saline (PBS) controls were included.

After 18 to 24 h of incubation at  $37^\circ\text{C}$  with 5% CO<sub>2</sub> and 98% humidity, the cells were examined for rounding. The endpoint of the titration was the last dilution showing  $\geq 50\%$  rounding of cells. Cells with toxin-negative filtrates or filtrates with *C. sordellii* antitoxin showed no rounding. The microtiter plates were incubated for another 24 h, and the results were then recorded.

The results of testing are shown in Table 1. Both McCoy and Vero cells detected *C. difficile* toxin in all 30 toxin-positive stool filtrates at  $\geq 1:100$  dilution. Tests were considered positive at  $\geq 1:100$  dilution, as previously described (6). MRC-5 cells detected toxin in 22 stool filtrates, while AGMK and RMK cells detected toxin in 16 and 10 stool filtrates, respectively. Donta et al. (4) have shown differences in toxin potency and cell sensitivity using mouse adrenal tumor, rat hepatoma, Chinese hamster ovary, and human cervical epithelium cells. Our results indicated that both McCoy and Vero cells were more sensitive to *C. difficile* toxin than were the other three cell lines tested. Hence, these two cell lines were selected for toxin assay of 630 stool specimens re-

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TABLE 1. Results of testing known positive stool filtrates

Endpoint dilution of filtrates	No. of toxin-positive stool filtrates detected by cell line:				
	McCoy	Vero	MRC-5	AGMK	RMK
1/50	0	0	2	4	6
1/100-1/200	9	12	9	8	6
1/500	8	5	5	4	1
1/1,000-1/2,000	10	11	8	4	3
1/4,000-1/8,000	3	2	0	0	0
Total of positive filtrates <sup>a</sup>	30/30	30/30	22/30	16/30	10/30
Negative results	0	0	8	14	20

<sup>a</sup> Positive filtrates are defined as those with cytotoxicity at endpoint dilutions of  $\geq 1/100$ . The total is represented by the number of positive filtrates/total number of filtrates tested.

ceived at Cadham Provincial Laboratory, Winnipeg, Manitoba, Canada, between April 1985 and April 1986. The results indicated that 70 (11.1%) stool filtrates were positive with both cell lines. There was 100% agreement between the two cell lines in detecting toxicity, both with positive filtrates and in terms of negative results, in these 630 consecutive clinical stool specimens. Borriello and Welch (1) reported similar results with their macro test, using monolayers of Vero cells grown in tubes.

McCoy and Vero cells showed cytotoxic effect within 24 h (toxic effect was often seen as early as 6 h after the addition

of toxin). MRC-5, AGMK, and RMK cells required up to 48 h to show toxic effect and failed to detect all positive specimens. Rounding of cells was marked in McCoy, Vero, and MRC-5 cells. MRC-5 cells showed rounding much more clearly than the remaining four cell lines did.

Vero cells gave results comparable to those for McCoy cells and offer a practical alternative for the rapid detection of *C. difficile* cytotoxin in fecal specimens.

#### LITERATURE CITED

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