The value of repeat *Clostridium difficile* toxin testing during and after an outbreak of *C difficile*-associated diarrhea

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BACKGROUND: The recent increase in *Clostridium difficile*-associated diarrhea (CDAD) has led to questions about the reproducibility and sensitivity of *C difficile* toxin testing (CDTT). While there have been recommendations to repeat CDTT following a negative result, previous studies have failed to show a benefit. However, no studies were performed during an outbreak of CDAD. The value of repeat CDTT after an initial negative result in patients tested during and after an outbreak of CDAD is reported in the present study, as well as the reproducibility of CDTT when multiple samples are received and tested on the same day.

METHODS: The results of CDTT, performed using a cell cytotoxicity assay between April 1, 2001, and March 31, 2008, were retrieved and searched for patients who had repeat samples tested after an initial negative result. The result and the number of days after a negative result were determined using the date of the most recent negative test. The cumulative positivity rate was calculated by adding all of the repeat positive test results for the days in question and dividing by the total number of tests performed during that time.

RESULTS: A total of 8661 patients submitted 14,991 stool specimens for CDTT during the study period. There were 3095 samples that tested positive (20.6%) for the toxin. The results were divided into two time periods to reflect the CDAD outbreak, which began in April 2002: period 1 (outbreak) was from April 1, 2002, to March 31, 2006, and period 2 was from April 1, 2006, to March 31, 2008. The rate of positivity was 24.2% during period 1, and 11.6% during period 2 (P<0.001). Repeat CDTT was performed 619 times on samples received on the same day as the initial specimen, and only three (0.5%) were discordant. A total of 1630 samples were retested within one to seven days of a negative result, and 103 (6.3%) tested positive (7.8% period 1 and 2.9% period 2; P<0.002). The likelihood of a positive result on repeat testing in the first three days after a negative result was low (0.9%, 7% and 4%, respectively). The cumulative positivity for repeat testing performed in the first three days was 0.9%, 3.3% and 3.5%, respectively, and did not differ significantly at day 3 during the period of high CDTT positivity (P=0.110).

CONCLUSIONS: The value of repeat CDTT, performed using a cell cytotoxicity assay, was low in the first three days after an initial negative result and was unchanged during a CDAD outbreak.

Key Words: *Clostridium difficile*; Toxin testing

There has been a worldwide increase in the incidence of *Clostridium difficile*-associated diarrhea (CDAD) (1). The toxins produced by *C difficile* can be detected using various methods including enzyme immunoassay (EIA), cell cytotoxicity assay (CTA) or polymerase chain reaction. There is some controversy regarding the sensitivity and specificity of the various toxin assays, which has led to the recommendation for repeat toxin testing at least once after a negative result (2) or use of a two-step method of detection, incorporating bacterial

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antigen or culture (3,4). Because the positivity rate for toxin detection is approximately 10% in nonoutbreak periods, this strategy could result in excessive and expensive repeat testing. Previous studies (5-12) investigating the value of repeat C difficile toxin testing (CDTT) have shown that between 0% and 11.2% of specimens that originally tested negative would test positive if repeated within five to 10 days of the first sample. The range in values may be attributed to the CDTT methodology used.

Beginning in 2002, the province of Quebec experienced a CDAD outbreak, with CDTT positivity rates approaching 30% (13). During this period, many microbiology laboratories received repeat specimens based on the clinical impression that the initial results were not accurate. There were anecdotal reports of specimens from patients with severe CDAD having initially negative CDTT results that were positive on repeat testing. This led physicians to request multiple stool samples that were sent on the same day or within a few days of the first sample. In one recent study (14), 57% of patients had more than one sample sent within a 24 h period. To examine the utility of repeat CDTT, the present study was designed to review all results over a continuous six-year period. Additionally, a comparison of repeat CDTT during and after a CDAD outbreak was performed.

METHODS
A Monarch data retrieval program was used to retrospectively search the laboratory results for all patients who had samples sent for CDTT between April 1, 2002, and March 31, 2008. During this period of time, all CDTT was performed solely by CTA using a standardized protocol. St Mary’s Hospital Center is a 316-bed McGill University-affiliated teaching hospital in Montreal, Quebec, that provides College of American Pathologists-accredited laboratory services for a predominantly adult population, with external clients that include several rehabilitation hospitals, a large mental health institution, more than 30 medical clinics and more than 300 community physicians. The vast majority of samples received for CDTT originate from this hospital and the institutions that it services. The date of sample testing was determined by the laboratory accession number, and this date was also used as the date of sample acquisition. The results were divided into two periods to reflect the CDAD outbreak that began at St Mary’s Hospital Center during the 2002/2003 fiscal year (Figure 1). Period 1 (outbreak) encompassed April 1, 2002, to March 31, 2006 (48 months) and period 2 from April 1, 2006, to March 31, 2008 (24 months). Patients who had an initial negative CDTT and underwent repeat testing anytime during the two study periods were examined. All results of patients with multiple initial samples tested on the same day were analyzed. Equivocal and weakly positive CDTT results were classified as positive results. Patients who had an initial negative CDTT result and had subsequent samples sent were studied. The number of days from the most recent negative result was determined. Once a positive result was obtained, no further samples were included in the analysis, with the exception of patients sending multiple samples on the same day.

Cumulative positivity was determined by adding the number of repeat positive test results by the total number of repeat tests performed over the indicated period. For example, the total number of repeat positive tests for days 1 and 2 was divided by the total number of repeat tests performed on days 1 and 2 to determine the cumulative repeat positivity result. Clinical information about the patients who were tested was not available. Two-tailed $\chi^2$ analysis and Fisher’s exact test were used to compare the different time periods.

RESULTS
Between April 1, 2002, and March 31, 2008, a total of 14,991 stool samples from 8661 patients were tested for C difficile toxin using a CTA. The testing results are summarized in Table 1. The overall positivity rate for the entire period was 20.6%, with a markedly higher rate during period 1 (outbreak) compared with period 2 (24.2% versus 11.6%, respectively; $P<0.001$). There were 619 samples received on the same day as the initial specimen. Repeat testing (Table 2) showed a discordance of only 0.5%. A total of 1630 samples were retested within one to seven days of a negative result (Figure 2). Of these, 103 (6.3%) tested positive (7.8% period 1 and 2.9% period 2; $P=0.002$). The likelihood of a positive result on repeat testing in the first three days after a negative result was low (0.9%, 7% and 4%, respectively). The cumulative positivity for repeat testing performed in the first three days was 0.9%, 3.3% and 3.5%, respectively (Figure 3). As expected, the percentage of positive repeat tests was higher during the outbreak period from days 4 to 7. There were 12.5% new positive results on day 7 (16.5% period 1 versus 4.7% period 2; $P=0.056$). The cumulative positivity rate for the seven days was 6.3% (8.5% period 1 versus 2.9% period 2; $P<0.001$).

DISCUSSION
The increased incidence of CDAD has been noted worldwide (1). During 2003, the province of Quebec was particularly affected, with a reported estimated quadrupling of cases from one region (13) – St Mary’s Hospital Center experienced a 10-fold increase in the number of nosocomial cases of CDAD (Figure 1). Establishing a diagnosis of CDAD usually requires the demonstration of toxin production in stool obtained from patients with diarrhea. Various methods have been used to perform CDTT; with each having some limitation. EIA testing lacks sensitivity when compared with CTAs, but has more rapid turnaround times (8). The current gold standard for CDTT is cultivation of stool for C difficile, followed by efforts to detect toxin production from the isolates (3).

This is labour intensive and time consuming. As a result, most clinical laboratories use EIA with or without initial screening for the

<table>
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<tr>
<th>TABLE 1</th>
<th>Results of Clostridium difficile toxin testing</th>
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<td></td>
<td>Patients, n</td>
</tr>
<tr>
<td>Period 1</td>
<td>5922</td>
</tr>
<tr>
<td>Period 2</td>
<td>2739</td>
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<td>Total</td>
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*P<0.001 compared with period 2
presence of C. difficile antigen. The knowledge that current CDTT may not detect all cases of CDAD has resulted in the recommendation that a repeat sample should be sent if the initial result is negative (2). Clinicians have also been uneasy relying on a single negative CDTT result for patients at risk.

This has led to numerous additional samples sent to the laboratory after an initial negative result (14). Other studies (4-6) using different methods have shown that there is little value in repeating CDTT within seven days of an initial result. In a smaller study (4) using CTA, the overall positivity rate was only 7.4%, and repeat testing of previously negative patients in the first seven days was positive in only two patients. A larger study (12) using EIA had a high initial positivity rate of 16.6%. A single repeat test was positive in 4.9% of samples, while a third test was positive in another 3%. These patients were studied for only five days per episode. Additional studies showed that repeat testing in the first three days after a negative result produced 1.8%, 3.8% and 2.6% new positives (8), while in another study (10), one in 78 (0.8%) initially negative repeat tests became positive. Although all these studies consistently show the minimal benefit of performing repeat testing after a negative result, none of the studies were performed during a CDAD outbreak. The present study compared CDTT during and after a major outbreak of CDAD at St Mary's Hospital Center. There was negligible benefit in repeating CDTT on specimens received on the same day. Only three of 619 (0.5%) samples repeated on the same day gave discordant results, which is a positive reflection on the reliability of the manner of testing. In addition, the laboratory has been accredited by the College of American Pathologists since 1995, and there have not been any discordant results while per-
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REFERENCES