JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2008, p. 3795–3797 Vol. 46, No. 11
0095-1137/08/$08.00+0 doi:10.1128/JCM.00684-08

Nonutility of Repeat Laboratory Testing for Detection of *Clostridium difficile* by Use of PCR or Enzyme Immunoassay▽

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Received 10 April 2008/Returned for modification 30 May 2008/Accepted 2 September 2008

The diagnostic gains of repeat testing for *Clostridium difficile* by enzyme immunoassay and PCR (i.e., initial negative result followed by positive result) within a 7-day period were 1.9 and 1.7%, respectively. There is little value of repeat testing for *C. difficile* by enzyme immunoassay or PCR.

Toxigenic culture and, to a lesser extent, cell culture cytotoxicity assay are the most sensitive methods for detection of *Clostridium difficile* (6). Because of rapidity, most laboratories use an enzyme immunoassay (EIA) to detect toxin A and/or B in stool. EIA has a lower sensitivity than that of toxigenic culture (14). American College of Gastroenterology practice guidelines state that "…when an EIA test, or other rapid test, is reported negative, it may then be worthwhile to send another stool the next day for testing by EIA or by different tests" (5).

In recent years, PCR has been shown to be a sensitive method for detection of *C. difficile* (2, 7, 12, 14–16). Recently, rapid PCR replaced EIA for *C. difficile* diagnosis at our institution. We determined the value of repeat testing for *C. difficile* by PCR versus EIA.

Results of stool testing for *C. difficile* from June 2006 through December 2007 were reviewed. Until 1 July 2007, EIA (Premier Toxins A&B assay; Meridian Bioscience Inc., Cincinnati, OH) was performed (14). A real-time PCR assay targeting *tcdC*, with a sensitivity of 86% and a specificity of 97% compared to toxigenic culture, was used after that time (14).

The data analyzed included the day the specimen was collected, patient age and gender, and test result. To detect repeat testing done on the same patient, tests were grouped into episodes, with an episode defined as one or more consecutive tests on one patient within a 7-day period. Subsequent testing on the same patient after this initial period was not analyzed.

Analyses were performed on the subsets of patients with exactly two tests, more than two tests, and finally, two or more tests in the initial 7-day period. Estimated positivity rates by test were reported as numbers (percentages), along with 95% exact binomial confidence intervals (95% CI). Positivity rates of the EIA and PCR tests for patients with an initial negative test were compared using the chi-square test. The alpha level was set at 0.05 for statistical significance.

C. difficile EIA or PCR test results were reported for 9,760 patients. Patients were excluded if they did not provide consent for review of their medical records (Minnesota statute 144.335) ($n = 365$) or were under the age of 16 ($n = 780$), leaving 5,788 patients tested by EIA and 2,827 patients tested by PCR. The mean age of patients in both groups was 59 years (median = 61 years), with ranges of 16 to 103 years for EIA and 16 to 97 years for PCR. Among the patients, 2,703 (46.7%) and 1,306 (46.2%) tested by EIA and PCR, respectively, were men. Of 4,313 subjects with only a single test by EIA, 333 (7.7%) had positive results. For PCR, this group consisted of 2,384 subjects, among whom 297 (12.5%) had positive results.

The group of EIA patients tested only twice consisted of 792 subjects (13.7% of patients tested with EIA) (Table 1). Six hundred eighty-three patients (11.8% of patients tested with EIA) had three or more EIA tests performed within 7 days (Tables 2 and 3), and 605 (88.6%) had only negative results (Table 2). Twenty (2.9%) patients had a negative result on the first test with subsequent positive results on the following tests (Table 2). The remaining 58 (8.5%) patients had a positive first test followed by subsequent positive or negative tests (Table 3).

Three hundred fifty-one patients were tested only twice by PCR (12.4% of patients tested by PCR) (Table 4). There were 92 patients (3.2% of patients tested by PCR) who had three or more PCR tests performed within 7 days. In 85 (92.4%) cases, results of all tests were negative. There were no patients who had positive results following an initial negative test. For six patients (6.5%), the results switched from an initial positive to a subsequent negative result, while one patient (1.1%) demonstrated only positive results (Table 5).

We compared the numbers of repeat EIA and PCR testing episodes where the result switched from being negative on the initial test to being positive over the course of multiple testing. We analyzed any patient who had two or more tests performed in 7 days, including all patients with exactly two tests and patients with three or more tests during this period. Of the 1,321 patients who had an initial negative EIA test, 25 (1.9%) were positive on the second test (95% CI, 1.2% to 2.7%),

TABLE 1. EIA results for patients with exactly two tests in 7 days ($n = 792$)

Result of test 1	Result of test 2	No. (%) of patients
Negative	Negative	708 (89.4)
Negative	Positive	13 (1.6)
Positive	Negative	38 (4.8)
Positive	Positive	33 (4.2)

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▽ Published ahead of print on 10 September 2008.

TABLE 2. EIA results for patients with three or more tests in 7 days and an initial negative result

Initial test result	Result of EIA within 7 days									No. of patients
	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8	Test 9	Test 10	
Negative	−	−								441
	−	−	−							108
	−	−	−	−						33
	−	−	−	−	−					13
	−	−	−	−	−	−				8
	−	−	−	−	−	−	−	−	−	2
	−	−	−	−	+					1
	−	−	+							3
	−	−	+	+						1
	−	+								2
	−	+	−	−						1
	+	−								4
	+	−	−							1
	+	−	−	−						1
	+	−	−	−	−					2
	+	−	+	−						1
	+	+								2
	+	+	+							1

TABLE 3. EIA results for patients with three or more tests in 7 days and an initial positive result

Initial test result	Results of EIA within 7 days										No. of patients
	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8	Test 9	Test 10	Test 11	
Positive	−	−									12
	−	−	−								8
	−	−	−	−							1
	−	−	−	−	−						1
	−	+									2
	+	−									9
	+	−	−								4
	+	−	−	−							1
	+	+									15
	+	+	−								2
	+	+	−	−							1
	+	+	+								1
	+	+	+	+	−	−	−	−	−	−	1

TABLE 4. PCR results for patients with exactly two tests in 7 days (n = 351)

Result 1	Result 2	No. (%) of patients
Negative	Negative	316 (90.0)
Negative	Positive	7 (2.0)
Positive	Negative	10 (2.9)
Positive	Positive	18 (5.1)

TABLE 5. PCR results for patients with three or more tests in 7 days and an initial positive result

Initial test result	Result of PCR within 7 days				No. of patients
	Test 2	Test 3	Test 4	Test 5	
+	−	−			1
+	−	−	−	−	1
+	+	−			4
+	+	+			1

compared to 1.7% (95% CI, 0.7% to 3.5%) becoming positive for the 401 patients having an initial negative PCR test (P = 1.0).

We studied, to the best of our knowledge, the largest group of patients analyzed for assessment of repeat *C. difficile* testing (Table 6) and demonstrated that the incremental yields of repeat testing by EIA and PCR are low and not statistically significantly different between the two tests. Several authors have suggested that it may be useful to test more than one stool specimen for *C. difficile* toxin by use of an immunoassay (1, 4, 11); this practice has been adopted widely in the clinical setting, including at our own institution. Nevertheless, there are limited data supporting this practice (Table 6). Since PCR is more sensitive than EIA (14), we expected to show a lower incremental yield of repeat testing for *C. difficile* via PCR versus EIA; this was not the case.

There are limitations to our study. EIA and PCR testing was done in sequential periods and consequently on different patient populations. The numbers of repeat tests performed varied between EIA and PCR, with 13.7% and 12.4% of the EIA and PCR groups, respectively, being tested just twice and 11.8% and 3.2% of the EIA and PCR groups, respectively, being tested three or more times ($P < 0.001$). The less frequent ordering of large numbers of PCR versus EIA tests may relate to laboratory practice changes made after implementation of PCR, allowing computer checking for repeated tests within 24 h and rejection of duplicate specimens.

TABLE 6. Selected studies evaluating repeat testing for *Clostridium difficile*

Reference[a]	Date	Test(s)	Total no. of patients/ no. of samples tested	No. of patients or samples with repeat testing	No. of tests converted from negative to positive
8	1995	EIA for toxins A and B (Cambridge Biotech, Worcester, MA), cell culture cytotoxicity assay	268/692	162	9
13	1996	Cell culture cytotoxicity assay	2,009/4,238	1,519	15
10	2004	EIA for toxins A and B (Meridian Bioscience Inc., Cincinnati, OH), PCR	130/147	63	1
3	2005	Cell culture cytotoxicity assay		1,101	2
9	2006	EIA for toxins A and B	396/474	78	1
16	2007	Enzyme-linked fluorescent assay (bioMérieux, Durham, NC), EIA for toxins A and B (Meridian Bioscience Inc., Cincinnati, OH), real-time PCR, cytotoxicity assay	450/547	68	2
Present report	2008	Premier toxin A and B assay (Meridian Bioscience Inc., Cincinnati, OH), real-time PCR	8,615/15,522	1,918	40

[a] Some cited studies may be underpowered.

We conclude that the diagnostic gains of repeat testing are equally low for PCR and EIA and that repeat testing for *C. difficile* should not be routine.

We thank Lynne M. Sloan and Jon E. Rosenblatt for their thoughtful reviews of the manuscript.

REFERENCES

1. **Bartlett, J. G.** 2002. Clinical practice. Antibiotic-associated diarrhea. N. Engl. J. Med. **346:**334–339.
2. **Belanger, S. D., M. Boissinot, N. Clairoux, F. J. Picard, and M. G. Bergeron.** 2003. Rapid detection of *Clostridium difficile* in feces by real-time PCR. J. Clin. Microbiol. **41:**730–734.
3. **Borek, A. P., D. Z. Aird, and K. C. Carroll.** 2005. Frequency of sample submission for optimal utilization of the cell culture cytotoxicity assay for detection of *Clostridium difficile* toxin. J. Clin. Microbiol. **43:**2994–2995.
4. **Debast, S. B., E. van Kregten, K. M. Oskam, T. van den Berg, R. J. Van den Berg, and E. J. Kuijper.** 2008. Effect on diagnostic yield of repeated stool testing during outbreaks of *Clostridium difficile*-associated disease. Clin. Microbiol. Infect. **14:**622–624.
5. **Fekety, R., et al.** 1997. Guidelines for the diagnosis and management of *Clostridium difficile*-associated diarrhea and colitis. Am. J. Gastroenterol. **92:**739–750.
6. **Gerding, D. N., S. Johnson, L. R. Peterson, M. E. Mulligan, and J. Silva, Jr.**1995. *Clostridium difficile*-associated diarrhea and colitis. Infect. Control Hosp. Epidemiol. **16:**459–477.
7. **Guilbault, C., A. C. Labbe, L. Poirier, L. Busque, C. Beliveau, and M. Laverdiere.** 2002. Development and evaluation of a PCR method for detection of the *Clostridium difficile* toxin B gene in stool specimens. J. Clin. Microbiol. **40:**2288–2290.
8. **Manabe, Y. C., J. M. Vinetz, R. D. Moore, C. Merz, P. Charache, and J. G. Bartlett.** 1995. *Clostridium difficile* colitis: an efficient clinical approach to diagnosis. Ann. Intern. Med. **123:**835–840.
9. **Mohan, S. S., B. P. McDermott, S. Parchuri, and B. A. Cunha.** 2006. Lack of value of repeat stool testing for *Clostridium difficile* toxin. Am. J. Med. **119:**356.e7–356.e8.
10. **Morelli, M. S., S. D. Rouster, R. A. Giannella, and K. E. Sherman.** 2004. Clinical application of polymerase chain reaction to diagnose *Clostridium difficile* in hospitalized patients with diarrhea. Clin. Gastroenterol. Hepatol. **2:**669–674.
11. **Moyenuddin, M., J. C. Williamson, and C. A. Ohl.** 2002. *Clostridium difficile*-associated diarrhea: current strategies for diagnosis and therapy. Curr. Gastroenterol. Rep. **4:**279–286.
12. **Peterson, L. R., R. U. Manson, S. M. Paule, D. M. Hacek, A. Robicsek, R. B. Thomson, Jr., and K. L. Kaul.** 2007. Detection of toxigenic *Clostridium difficile* in stool samples by real-time polymerase chain reaction for the diagnosis of *C. difficile*-associated diarrhea. Clin. Infect. Dis. **45:**1152–1160.
13. **Renshaw, A. A., J. M. Stelling, and M. H. Doolittle.** 1996. The lack of value of repeated *Clostridium difficile* cytotoxicity assays. Arch. Pathol. Lab. Med. **120:**49–52.
14. **Sloan, L. M., B. J. Duresko, D. R. Gustafson, and J. E. Rosenblatt.** 2008. Comparison of real-time PCR for detection of the *tcdC* gene with four toxin immunoassays and culture in diagnosis of *Clostridium difficile* infection. J. Clin. Microbiol. **46:**1996–2001.
15. **van den Berg, R. J., L. S. Bruijnesteijn van Coppenraet, H. J. Gerritsen, H. P. Endtz, E. R. van der Vorm, and E. J. Kuijper.** 2005. Prospective multicenter evaluation of a new immunoassay and real-time PCR for rapid diagnosis of *Clostridium difficile*-associated diarrhea in hospitalized patients. J. Clin. Microbiol. **43:**5338–5340.
16. **van den Berg, R. J., N. Vaessen, H. P. Endtz, T. Schulin, E. R. van der Vorm, and E. J. Kuijper.** 2007. Evaluation of real-time PCR and conventional diagnostic methods for the detection of *Clostridium difficile*-associated diarrhoea in a prospective multicentre study. J. Med. Microbiol. **56:**36–42.