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Impact of Changes in *Clostridium difficile* Testing Practices on Stool Rejection Policies and *C. difficile* Positivity Rates across Multiple Laboratories in the United States

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We describe the adoption of nucleic acid amplification tests (NAAT) for *Clostridium difficile* diagnosis and their impact on stool rejection policies and *C. difficile* positivity rates. Of the laboratories with complete surveys, 51 (43%) reported using NAAT in 2011. Laboratories using NAAT had stricter rejection policies and increased positivity rates.

Clostridium difficile infection (CDI) continues to be an important public health problem due to its high incidence, morbidity, and medical care costs (1, 2). Rapid and reliable *C. difficile* diagnosis is an important component in preventing transmission of *C. difficile* (2–4). The traditional toxin enzyme immunoassays (EIA) used by many clinical laboratories are known to have a low sensitivity; however, they have been widely adopted due to their simplicity, their low cost, their rapid turnaround time, and, until recently, lack of a better alternative (4, 5). Since 2008, the U.S. Food and Drug Administration has approved eight nucleic acid amplification tests (NAAT) for *C. difficile* testing (6). These NAAT have sensitivities ranging from 84% to 94% and short turnaround times compared with toxigenic stool culture, making them attractive to clinical laboratories (7, 8). Although laboratory practice guidelines discourage repeat *C. difficile* testing and testing of formed stools regardless of the assay type (9–12), it is unclear how well these guidelines have been adopted by U.S. clinical laboratories and if the introduction of NAAT has resulted in changes to stool rejection policies.

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From 1 November 2011 to 31 January 2012, we surveyed 121 laboratories serving 11.2 million people in 10 U.S. states participating in the CDC's Emerging Infections Program population-based CDI surveillance (13). We compared stool rejection policies of laboratories using NAAT to those of laboratories that do not, assessed changes in stool rejection policies after NAAT implementation, and evaluated the impact of NAAT adoption on *C. difficile* positivity rates. A NAAT laboratory was defined as a laboratory using NAAT as a first- or second-line test. A non-NAAT laboratory was defined as a laboratory using assays other than NAAT, such as toxin EIA, glutamate dehydrogenase (GDH), cell cytotoxicity neutralization assays, or toxigenic culture. Data collection included current testing practices, changes to testing algorithms and stool rejection policies in the past year, and the number of stool specimens tested and the number of stools positive for *C. difficile* in the 3 months before and after NAAT implementation. The chi-square test and the Wilcoxon signed-rank test were used to evaluate differences.

Surveys were completed by 120 (99%) of the 121 laboratories surveyed, representing 88 inpatient and 32 outpatient laboratories. Fifty-one (43%) laboratories reported using NAAT; of these, 27 (53%) switched to NAAT as either a first-line ($n = 20$) or second-line ($n = 7$) test in 2011. Prior to the switch, 24 (89%) laboratories used EIA while 3 used GDH plus EIA. Among the 69 laboratories not reporting NAAT use, 50 (72%) used EIA, 13 (19%) used GDH plus EIA, 2 (3%) used GDH only, and 4 (6%) used other testing methods. Among all laboratories, those using NAAT were more likely to reject formed stools (88% versus 54%; $P < 0.0001$) and to not repeat *C. difficile* stool testing within 48 h after a negative specimen (14% versus 0%; $P = 0.001$) compared to non-NAAT laboratories; 98% of NAAT laboratories reported having rejection policies, compared to 84% of non-NAAT laboratories ($P = 0.01$). Of the 27 laboratories that switched to NAAT in 2011, 23 (85%) implemented more-stringent policies after the switch, such as restriction of testing of multiple specimens within 48 h ($n = 15$) and/or rejection of formed stools ($n = 13$). Data on the number of stool specimens submitted for *C. difficile* testing and the number positive in the 3 months prior to and following a switch to NAAT were available for 18 of 27 laboratories (Table 1). Among the 13 laboratories that switched to NAAT as a first-line test, the mean number of specimens submitted for testing decreased from 365 prior to the switch to 301 after the switch ($P < 0.001$), while the percentage of positive specimens increased from

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TABLE 1 Changes in the number of stool specimens tested for *C. difficile* and the percentage of positive specimens by laboratory after implementation of NAAT

Testing algorithm and laboratory no. (previous method)	Mean no. (range) of specimens tested			Mean no. (%) of positive specimens		
	3 mos before switch	3 mos after switch	*P* value[a]	3 mos before switch	3 mos after switch	*P* value[b]
NAAT (implementation as first-line test)[c]						
1 (EIA)	271 (225–342)	229 (213–255)	0.21	55 (20.4)	75 (33.0)	0.001
2 (EIA)	125 (124–129)	94 (80–108)	0.06	10 (8.2)	18 (20.2)	0.01
3 (EIA)	446 (400–515)	358 (322–396)	0.24	39 (8.9)	57 (16.0)	0.001
4 (GDH plus EIA)	921 (914–929)	1,013 (976–1,045)	0.05	268 (29.1)	200 (19.8)	<0.001
5 (EIA)	171 (147–206)	99 (95–106)	0.05	12 (7.2)	18 (18.4)	0.006
6 (EIA)	717 (700–734)	500 (466–544)	0.006	73 (10.2)	93 (18.6)	<0.001
7 (GDH plus EIA)	319 (260–350)	357 (342–366)	0.38	22 (6.9)	88 (24.7)	<0.001
8 (EIA)	568 (539–596)	404 (389–414)	0.004	42 (7.4)	64 (16.0)	<0.001
9 (EIA)	91 (54–126)	75 (72–78)	0.54	10 (11.6)	7 (9.7)	0.73
10 (EIA)	483 (426–542)	240 (228–253)	0.01	70 (14.5)	59 (24.8)	0.001
11 (EIA)	77 (59–90)	57 (56–58)	0.17	9 (12.0)	11 (19.7)	0.23
12 (EIA)	384 (367–405)	358 (341–391)	0.41	46 (12.1)	81 (22.8)	<0.001
13 (EIA)	173 (163–180)	129 (127–132)	0.01	15 (8.9)	23 (18.0)	0.01
Total	365 (54–929)	301 (56–1,045)	<0.001	51 (14.0)	61 (20.3)	0.03
NAAT following GDH plus EIA (implementation as second-line test)[d]						
14 (EIA)	398 (382–416)	374 (361–389)	0.10	40 (10.1)	39 (10.6)	0.86
15 (EIA)	887 (877–891)	660 (528–732)	0.06	100 (11.3)	117 (17.8)	<0.001
16 (GDH plus EIA)	46 (28–70)	39 (24–51)	0.68	6 (13.7)	9 (23.1)	0.24
17 (EIA)	76 (47–103)	97 (95–100)	0.27	13 (17.1)	16 (17.1)	0.91
Total	352 (28–891)	292 (24–732)	0.09	40 (11.4)	45 (15.4)	0.13
NAAT following GDH (implementation as second-line test)						
18 (EIA)	3,967 (3,743–4,371)	3,453 (3,395–3,514)	0.09	397 (10.0)	401 (11.6)	0.02

[a] By Wilcoxon signed-rank test.
[b] By Chi-square test.
[c] Seven laboratories that switched to NAAT as a first-line test did not report the number of stool specimens tested and the number of stools positive in the 3 months before and after NAAT implementation.
[d] Two laboratories that switched to NAAT as a second-line test did not report the number of stool specimens tested and the number of stools positive in the 3 months before and after NAAT implementation.

14.0% prior to the switch to 20.3% afterwards ($P = 0.03$) (Table 1). Although not statistically significant, a decrease in the number of specimens tested was observed among laboratories that implemented NAAT as a reflex test after a 2-step algorithm of GDH plus EIA; the mean number of specimens tested decreased from 352 before the switch to 292 after the switch ($P = 0.09$). No significant increase in the overall positivity rates was observed (11.4% prior to the switch versus 15.4% after the switch; $P = 0.13$) (Table 1). Only one laboratory implemented NAAT as a confirmatory test following GDH. This laboratory did not have a significant decrease in the mean number of specimens tested (3,967 prior to the switch and 3,453 after the switch; $P = 0.09$) but had a significant increase in the percentage of positive specimens (10.0% versus 11.6%; $P = 0.02$).

Early *C. difficile* diagnosis is critical for clinical management and prevention of further disease transmission. Our survey indicates that more laboratories are including NAAT as part of their routine CDI testing algorithms, a practice which will improve *C. difficile* detection, especially when NAAT are used as first-line tests. Laboratories switching to NAAT as first-line tests had a mean increase of 45% (−31.9% to 258.5%) in *C. difficile* positivity rates after the switch. The implementation of a multistep algorithm involving NAAT was reported to be associated with an increased positivity rate of 110%, from 4.7% to 9.9%, in a hospital previously using GDH plus EIA (14) and 97%, from 2.5% to 5.6%, in another hospital previously using EIA (15). We did not, however, observe an increase in the overall proportion of positive specimens among laboratories using NAAT following GDH plus EIA, with the exception of one laboratory that was using EIA only before the switch and observed an increased positivity rate of 57%. Although it is unclear why laboratories did not observe an increase in positivity rates after implementation of a multistep algorithm involving NAAT, one possible explanation may be the relatively low number of specimens processed by these laboratories and our short observation period.

Concerns about detection of colonized patients with NAAT have been raised and emphasize the importance of testing patients with clinically significant diarrhea in order to avoid false-positive tests and unnecessary treatments (16–18). In our study, the majority (85%) of the laboratories adopted more-stringent stool rejection policies after NAAT implementation. While we did not specifically ask laboratories to explain the rationale for adopting more-stringent policies, it is possible that the increased sensitivity of NAAT, its rapid turnaround time, and its initial high cost were contributory factors. We also observed decreases in the number of stools being tested by the laboratories after NAAT implementa-

tion (Table 1). Although more-stringent stool rejection policies likely led to these declines, other reasons, such as fewer stools per patient being collected due to the rapid turnaround and high sensitivity of NAAT, should be considered.

The study has several limitations. The sample of laboratories was not chosen to be representative of all U.S. laboratories, and some testing algorithms were relatively uncommon; both of these make the generalizability of these findings somewhat limited.

It is likely that laboratories will continue adopting NAAT as part of their routine testing methods due to their higher sensitivity, rapid turnaround, and improved detection of *C. difficile*. NAAT implementation will likely improve compliance with recommended stool rejection policies (i.e., no formed stool, no test for cure, and no multiple testing within 7 days of a negative specimen), improve detection, and potentially reduce the use of laboratory resources by requiring fewer tests.

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