AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

COMMENTARY

Strategies for Optimizing the Diagnostic Predictive Value of *Clostridium difficile* Molecular Diagnostics

Larry K. Kociolek

Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA; Division of Infectious Diseases, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois, USA

ABSTRACT Because nucleic acid amplification tests (NAATs) do not distinguish *Clostridium difficile* infection (CDI) and asymptomatic *C. difficile* carriage, the diagnostic predictive value of NAATs is limited when used in patients with a low probability of CDI. In this issue of the *Journal of Clinical Microbiology*, Truong et al. (J. Clin. Microbiol., 55:1276–1284, 2017, https://doi.org/10.1128/JCM.02319-16) report significant reductions in hospital-onset CDI and oral vancomycin utilization at their institution following implementation of a novel intervention that leveraged their clinical bioinformatics resources to prevent *C. difficile* testing of stools from patients without clinically significant diarrhea and in patients with recent laxative use.

Accepted manuscript posted online 8 March 2017

Citation Kociolek LK. 2017. Strategies for optimizing the diagnostic predictive value of *Clostridium difficile* molecular diagnostics. J Clin Microbiol 55:1244–1248. https://doi.org/10.1128/JCM.00147-17.

Editor Carey-Ann D. Burnham, Washington University School of Medicine

Address correspondence to lkociolek@luriechildrens.org.

For the article discussed, see https://doi.org/10.1128/JCM.02319-16.

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.

In recent years, molecular diagnostic tests have been developed and commercialized for a vast array of infectious diseases (1). Many clinical microbiology laboratories now rely on molecular diagnostic tests as the primary method for the detection of many pathogens, particularly for viruses and for bacteria that are difficult to cultivate. Molecular diagnostic tests generally provide rapid results, have improved sensitivity, may offer the ability to identify several pathogens with a single multiplex assay, and augment clinical microbiology laboratory efficiency by reducing technologist labor time for individual tests. Cost can be a significant barrier to implementation, but as the variety of assays continues to expand, equipment and staff training become increasingly more cost-effective. Thus, there are business incentives for manufacturers to expand their selection of diagnostic test offerings and cost incentives for laboratories to utilize them.

The exquisite sensitivity of many molecular diagnostic tests presents a challenge to the clinician interpreting the test result, and as the variety of assays from individual manufacturers continues to expand, this challenge becomes more common and complex. The clinical significance of a positive result for a highly sensitive diagnostic test may be unclear. For example, detection of a respiratory or gastrointestinal virus may occur in the absence of symptoms related to that infection (i.e., a subclinical illness) or represent prolonged shedding following resolution of a previous illness. Thus, the pathogen detected may not be the true cause of the patient's illness, and mistakenly attributing the patient's illness to the detected pathogen may adversely impact patient care. For example, the patient may suffer adverse effects of unnecessary treatment or of initiated hospital isolation precautions. Additionally, the test result may preclude evaluation for the correct etiology of the patient's illness.

Clostridium difficile is an excellent example of a pathogen for which molecular diagnostics have overcome a variety of challenges associated with other diagnostic modalities (2). Toxigenic strains of *C. difficile*, an obligate anaerobic bacterium, cause disease through the expression of toxins, primarily toxin B. Toxin production usually occurs in the setting of intestinal microbiota perturbations resulting from antibiotic exposure (3). However, asymptomatic carriage of toxigenic strains can occur in a host

with normal protective intestinal microbiota and/or in patients with protective immunity against *C. difficile* toxins and potentially against non-toxin antigens that had developed following previous exposure to *C. difficile* (4).

Because *C. difficile* toxin expression is the primary driver of *C. difficile* infection (CDI) symptomatology, the most specific *C. difficile* diagnostic tests detect free toxin in stool (2). The gold standard for toxin detection is the cell culture cytotoxicity neutralization assay (CCCNA). In this assay, an appropriate cell line is observed for cytopathic effect (CPE) after incubation with a fecal filtrate prepared from a clinical stool specimen. In cases in which CPE is observed, a positive test is confirmed if CPE is prevented with *C. difficile* (or *C. sordelii*) antiserum. Because of technical difficulties with this assay, long turnaround times, and potentially suboptimal sensitivity compared to that of toxigenic stool culture, CCCNA is not commonly performed as a routine diagnostic test.

Toxigenic culture is considered the gold standard for detecting a toxigenic strain of *C. difficile* in a stool specimen (2). First, *C. difficile* is cultured anaerobically. To differentiate between a toxigenic strain and a nontoxigenic strain of *C. difficile*, the broth supernatant is assessed for the presence of toxin, either by CCCNA or toxin enzyme immunoassay (EIA). Thus, toxigenic culture identifies *C. difficile* toxin production only *in vitro*, identifying a strain that has the potential to produce toxin *in vivo*. This is in contrast to CCCNA, which identifies toxin production *in vivo*. Similarly to CCCNA, toxigenic culture suffers from long turnaround time and relatively extensive labor requirements that limit its use for routine diagnostic testing. Furthermore, because toxigenic culture does not identify the toxin *in vivo*, it does not reliably differentiate between CDI and asymptomatic *C. difficile* carriage. Toxigenic culture is considered to have greater sensitivity than CCCNA and is thus generally considered to be the preferred test between these two gold standards (2). Although stools that test toxigenic culture positive and CCCNA negative could potentially represent a false-negative CCCNA result, similar results could also occur in patients with asymptomatic *C. difficile* carriage. Thus, studies assessing CDI test performance are most reliable when ensuring that stools are collected from patients who meet the clinical definition of CDI (5).

Because of long turnaround times and labor requirements for CCCNA and toxigenic culture, neither of the tests is commonly performed clinically. Toxin EIAs represent the most commonly utilized commercially available assay for the detection of free toxin in stool (2). Toxin EIAs have fallen out of favor in the United States because of perceived suboptimal sensitivity (i.e., frequent false-negative results) (6). However, closer examination of previous studies assessing performance of toxin EIAs demonstrates that toxin EIA sensitivity is generally higher in testing against CCCNA, the gold standard for toxin production, than against toxigenic culture, which does not detect free toxin in stool (2). Similarly to the concerns regarding CCCNA sensitivity described above, stools that test toxigenic culture positive and toxin EIA negative could represent asymptomatic *C. difficile* carriage rather than a falsely negative EIA result.

Nonetheless, skepticism regarding the sensitivity of toxin EIAs for the diagnosis of CDI in the United States is prevalent. Nucleic acid amplification tests (NAATs), such as PCR and loop-mediated isothermal amplification (LAMP), are now the most commonly used tests for the diagnosis of CDI in the United States (7, 8). The excellent sensitivity and analytical specificity, low labor requirements, and quick turnaround time of NAATs have prompted the widespread adoption of NAATs as the preferred method of CDI diagnosis (2, 6). The exquisite sensitivity of NAATs is supported by the substantial increases in CDI rates across medical centers after the transition from toxin EIAs to NAATs for CDI diagnosis (2, 6, 9).

Based on existing sensitivity and analytical specificity data for NAATs and toxin EIAs, one could argue that NAATs are superior because they provide a rapid and more reliable method of CDI diagnosis, which expedites the initiation of appropriate treatment and prompt early isolation of infected patients to limit *C. difficile* hospital transmission. However, numerous studies have raised concerns about the clinical significance of positive results from *C. difficile* NAATs. NAATs primarily detect *tcdB*, the gene that encodes toxin B, and some assays additionally detect *tcdA*. Thus, like toxigenic

culture, NAATs identify a strain in stool that has the potential to produce toxin *in vivo*. Two large clinical research studies in the United States (10) and the United Kingdom (11) questioned the clinical significance of a positive PCR test (10) (or toxigenic culture [11]) when the patient also tests negative by toxin EIA because these patients have benign clinical outcomes that are similar to those seen with patients who do not have CDI. Furthermore, several studies have demonstrated the high rate of NAAT (or toxigenic culture) positivity in patients without diarrhea, including hospitalized children (12) and adults (9) as well as children with cancer (13). In addition, even among patients with diarrhea, a high proportion test positive by NAAT who have a likely alternate diarrheal etiology, such as recent laxative use (2) or a concomitant viral illness (14). With the recent incorporation of toxigenic *C. difficile* testing in multiplex PCR panels that can detect several gastrointestinal pathogens with a single assay, *C. difficile* has become the most common pathogen that is detected concomitantly with other pathogens (15, 16).

The plethora of data demonstrating a high frequency of *C. difficile* NAAT positivity in patients without CDI reveals the primary limitation of NAATs for CDI diagnosis: substantial discordance between analytical specificity and diagnostic specificity. While the analytical specificity of a diagnostic test refers to the accurate detection of a substance in a sample (irrespective of the clinical condition of the patient), diagnostic specificity refers to the accurate diagnosis of a patient with the particular condition (17). Analytical specificity and diagnostic specificity diverge when a large proportion of samples collected from patients without the clinical condition contain the target pathogen. Under such circumstances, the diagnostic predictive value is suboptimal when the test is used in patients who are unlikely to have an infection related to the target pathogen. A well-described example of a test with divergent analytical and diagnostic specificities is the group A streptococcal rapid antigen detection test (RADT) (18). Because RADTs have excellent analytical specificity, they also accurately identify patients who have pharyngeal streptococcal carriage. The frequency of asymptomatic group A streptococcal carriage in school-aged children is up to 20 to 30%. Thus, carriers of group A streptococcus who have a viral upper respiratory infection test positive by RADT, likely leading to unnecessary treatment for streptococcal pharyngitis. Because the positive predictive value is increased when the test is used in a high-prevalence population, the Centor criteria were established to guide testing decisions for streptococcal pharyngitis and to encourage physicians to limit testing to only those with a high pretest probability of harboring the infection.

Although similar formal criteria for CDI testing do not exist, limiting CDI testing in low-risk populations can augment the diagnostic predictive value of *C. difficile* NAATs and reduce misdiagnosis of CDI in patients with *C. difficile* carriage who do not have clinically significant diarrhea and/or whose diarrhea is more likely to be related to an alternate etiology. This is exceedingly important in particular patient populations, such as hospitalized children (12) and adults (9), in whom the prevalence of *C. difficile* carriage exceeds 10 to 20%, and children with cancer, in whom carriage rates may be as high as 30 to 50% (13). However, because hospitalization and malignancy, for example, can be risk factors for both CDI and *C. difficile* carriage, *C. difficile* testing decisions can be quite difficult in practice.

In this issue of *Journal of Clinical Microbiology*, Truong and colleagues (19) report their results from a novel intervention that leveraged their clinical bioinformatics resources to prevent *C. difficile* PCR testing of stools from patients without clinically significant diarrhea and from patients with recent laxative use. Nursing staff members were trained to record the consistency of all stools in the Epic (Verona, WI) electronic health record (EHR). In the EHR, a real-time data tracking report was developed to demonstrate dates/times of bowel movements, stool consistency, and recent laxative administration. When *C. difficile* PCR was ordered by a clinician, laboratory personnel reviewed this report and cancelled tests for patients who did not meet criteria for clinically significant diarrhea related to CDI (i.e., patients with <3 unformed/mucous stools in 24 h) (6) and/or in those who received laxatives in the previous 48 h. This intervention resulted in a significant reduction of hospital-onset CDI rates, as well as of

the frequency of oral vancomycin utilization. Notably, the intervention was safe; there was no difference in complication rates between patients with cancelled CDI testing orders and patients with clinically significant diarrhea who tested negative for *C. difficile*.

This novel intervention is an evidence-based, potentially broadly reproducible approach that can supplement many other strategies for reducing unnecessary CDI testing. While many laboratories currently perform *C. difficile* testing only on unformed stools (i.e., stools that take the shape of the container), the intervention devised by Truong and colleagues would additionally limit testing on stools that are unformed as a consequence of laxative use or that are collected from a patient with fewer than 3 unformed stools in a 24-h period. Another strategy for limiting unnecessary *C. difficile* testing includes avoiding repeat NAATs during the same diarrheal illness. As an exquisitely sensitive assay that can reliably rule out CDI with a single test, the diagnostic predictive value of NAATs drops significantly with each subsequent test once the patient initially tests negative. Many children's hospitals limit *C. difficile* testing to only children 12 months of age or older to avoid misdiagnosis of CDI in infants (8), a population with a high frequency of *C. difficile* carriage and in whom symptomatic CDI almost never occurs. In hospitals that have transitioned to a multiplex PCR platform for the detection of several diarrheal pathogens in a single assay, to avoid misdiagnosis of CDI in patients with *C. difficile* carriage, some suppress the *C. difficile* result unless the result is requested by the clinician.

While the intervention devised by Truong and colleagues was very effective in the hospital setting, it cannot be implemented in patients being evaluated for a diarrheal illness in the outpatient setting. Hospital-onset CDI represents only a minority of CDIs in pediatric and adult populations (20, 21). Furthermore, in our pediatric population, inappropriate *C. difficile* testing was performed frequently in children with community-onset diarrhea (14). Based on these data at our children's hospital, we implemented an intervention incorporating education of health care providers regarding the limitations of NAATs in children and an EHR alert to clinicians ordering *C. difficile* PCR testing (22). This alert cautioned against ordering *C. difficile* PCR in children at low risk of CDI, such as those in the following categories: children without CDI risk factors and with vomiting as a significant complaint (i.e., children more likely to have a viral diarrheal illness); children without clinically significant diarrhea; children with recent laxative use; children whose diarrhea resolved after recent treatment (i.e., avoiding "test of cure"); and children with a negative PCR result from the previous 7 days. During the 18 months following the intervention, we demonstrated a significant reduction in *C. difficile* PCR testing and test positivity rates (particularly in the outpatient and emergency department setting) and a reduction of approximately $250,000 in patient charges related to unnecessary *C. difficile* testing and subsequent treatment. Thus, similarly to the intervention developed by Truong and colleagues, our testing intervention was also important for CDI-related antibiotic stewardship.

In summary, there is an abundance of evidence suggesting that inappropriate use of *C. difficile* NAATs in low-risk populations reduces the assay diagnostic predictive value and leads to CDI misdiagnosis and unnecessary treatment. Furthermore, because health care-associated infection (HAI) rates are an important hospital quality metric, and with the threat of hospital nonreimbursement for HAIs such as CDI, hospitals have a financial incentive for accurately measuring and avoiding overestimation of CDI rates (23). Prudent use of NAATs through health care provider education, clinical microbiology laboratory oversight, and leveraging of hospital bioinformatics resources can optimize the diagnostic predictive value of *C. difficile* NAATs. Ideally, assays that can more reliably differentiate patients with *C. difficile* carriage from those with CDI are necessary to completely overcome the discordant analytical and diagnostic specificities of NAATs. An ultrasensitive toxin enzyme-linked immunosorbent assay currently under clinical investigation may be one such assay that provides improved diagnostic specificity while overcoming the potentially poor sensitivity of existing toxin EIAs (24). However, even if this important diagnostic challenge for CDI is solved with improved

diagnostics, highly sensitive molecular diagnostics will continue to emerge and be more broadly utilized for other infectious diseases. Thus, implementation and utilization of these assays in a manner that limits misdiagnosis will be vitally important for optimizing the clinical utility of molecular diagnostic assays.

ACKNOWLEDGMENTS

I have received research grant support from Merck and research support (supplies) from Alere/Techlab and have been a scientific advisor for Actelion.

REFERENCES

1. Emmadi R, Boonyaratanakornkit JB, Selvarangan R, Shyamala V, Zimmer BL, Williams L, Bryant B, Schutzbank T, Schoonmaker MM, Amos Wilson JA, Hall L, Pancholi P, Bernard K. 2011. Molecular methods and platforms for infectious diseases testing: a review of FDA-approved and cleared assays. J Mol Diagn 13:583–604. https://doi.org/10.1016/j.jmoldx.2011.05.011.
2. Burnham CA, Carroll KC. 2013. Diagnosis of Clostridium difficile infection: an ongoing conundrum for clinicians and for clinical laboratories. Clin Microbiol Rev 26:604–630. https://doi.org/10.1128/CMR.00016-13.
3. Kociolek LK, Gerding DN. 2016. Breakthroughs in the treatment and prevention of Clostridium difficile infections. Nat Rev Gastroenterol Hepatol 13:150–160. https://doi.org/10.1038/nrgastro.2015.220.
4. Kelly CP, Kyne L. 2011. The host immune response to Clostridium difficile. J Med Microbiol 60:1070–1079. https://doi.org/10.1099/jmm.0.030015-0.
5. Dubberke ER, Han Z, Bobo L, Hink T, Lawrence B, Copper S, Hoppe-Bauer J, Burnham CA, Dunne WM, Jr. 2011. Impact of clinical symptoms on interpretation of diagnostic assays for Clostridium difficile infections. J Clin Microbiol 49:2887–2893. https://doi.org/10.1128/JCM.00891-11.
6. Cohen SH, Gerding DN, Johnson S, Kelly CP, Loo VG, McDonald LC, Pepin J, Wilcox MH; Society for Healthcare Epidemiology of America; Infectious Diseases Society of America. 2010. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). Infect Control Hosp Epidemiol 31:431–455. https://doi.org/10.1086/651706.
7. Wong KK, Choi B, Fraser TG, Donskey CJ, Deshpande A. 28 October 2016. Diagnostic testing methods for Clostridium difficile infection: a statewide survey of Ohio acute care hospitals. Am J Infect Control https://doi.org/10.1016/j.ajic.2016.09.007.
8. Kociolek LK, Sandora TJ. 2013. National variability in surveillance, testing, and infection prevention for Clostridium difficile infection in pediatric populations. Am J Infect Control 41:933–935. https://doi.org/10.1016/j.ajic.2013.02.020.
9. Koo HL, Van JN, Zhao M, Ye X, Revell PA, Jiang ZD, Grimes CZ, Koo DC, Lasco T, Kozinetz CA, Garey KW, DuPont HL. 2014. Real-time polymerase chain reaction detection of asymptomatic Clostridium difficile colonization and rising C. difficile-associated disease rates. Infect Control Hosp Epidemiol 35:667–673. https://doi.org/10.1086/676433.
10. Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, Nguyen HH, Huang B, Tang YW, Lee LW, Kim K, Taylor S, Romano PS, Panacek EA, Goodell PB, Solnick JV, Cohen SH. 2015. Overdiagnosis of Clostridium difficile Infection in the molecular test era. JAMA Intern Med 175:1792–1801. https://doi.org/10.1001/jamainternmed.2015.4114.
11. Planche TD, Davies KA, Coen PG, Finney JM, Monahan IM, Morris KA, O'Connor L, Oakley SJ, Pope CF, Wren MW, Shetty NP, Crook DW, Wilcox MH. 2013. Differences in outcome according to Clostridium difficile testing method: a prospective multicentre diagnostic validation study of C difficile infection. Lancet Infect Dis 13:936–945. https://doi.org/10.1016/S1473-3099(13)70200-7.
12. Leibowitz J, Soma VL, Rosen L, Ginocchio CC, Rubin LG. 2015. Similar proportions of stool specimens from hospitalized children with and without diarrhea test positive for Clostridium difficile. Pediatr Infect Dis J 34:261–266. https://doi.org/10.1097/INF.0000000000000556.
13. Dominguez SR, Dolan SA, West K, Dantes RB, Epson E, Friedman D, Littlehorn CA, Arms LE, Walton K, Servetar E, Frank DN, Kotter CV, Dowell E, Gould CV, Hilden JM, Todd JK. 2014. High colonization rate and prolonged shedding of Clostridium difficile in pediatric oncology patients. Clin Infect Dis 59:401–403. https://doi.org/10.1093/cid/ciu302.
14. Kociolek LK, Patel SJ, Zheng X, Todd KM, Shulman ST, Gerding DN. 2016. Clinical and microbiologic assessment of cases of pediatric community-associated Clostridium difficile infection reveals opportunities for improved testing decisions. Pediatr Infect Dis J 35:157–161. https://doi.org/10.1097/INF.0000000000000954.
15. Nicholson MR, Van Horn GT, Tang YW, Vinje J, Payne DC, Edwards KM, Chappell JD. 2016. Using multiplex molecular testing to determine the etiology of acute gastroenteritis in children. J Pediatr 176:50–56.e52. https://doi.org/10.1016/j.jpeds.2016.05.068.
16. Khare R, Espy MJ, Cebelinski E, Boxrud D, Sloan LM, Cunningham SA, Pritt BS, Patel R, Binnicker MJ. 2014. Comparative evaluation of two commercial multiplex panels for detection of gastrointestinal pathogens by use of clinical stool specimens. J Clin Microbiol 52:3667–3673. https://doi.org/10.1128/JCM.01637-14.
17. Saah AJ, Hoover DR. 1997. "Sensitivity" and "specificity" reconsidered: the meaning of these terms in analytical and diagnostic settings. Ann Intern Med 126:91–94. https://doi.org/10.7326/0003-4819-126-1-199701010-00026.
18. Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan EL, Lee G, Martin JM, Van Beneden C. 2012. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. Clin Infect Dis 55:1279–1282. https://doi.org/10.1093/cid/cis847.
19. Truong CY, Gombar S, Wilson R, Sundararajan G, Tekic N, Holubar M, Shepard J, Madison A, Tompkins L, Shah N, Deresinski S, Schroeder LF, Banaei N. 2017. Real-time electronic tracking of diarrheal episodes and laxative therapy enables verification of *Clostridium difficile* clinical testing criteria and reduction of *Clostridium difficile* infection rates. J Clin Microbiol 55:1276–1284. https://doi.org/10.1128/JCM.02319-16.
20. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, Farley MM, Holzbauer SM, Meek JI, Phipps EC, Wilson LE, Winston LG, Cohen JA, Limbago BM, Fridkin SK, Gerding DN, McDonald LC. 2015. Burden of Clostridium difficile infection in the United States. N Engl J Med 372:825–834. https://doi.org/10.1056/NEJMoa1408913.
21. Wendt JM, Cohen JA, Mu Y, Dumyati GK, Dunn JR, Holzbauer SM, Winston LG, Johnston HL, Meek JI, Farley MM, Wilson LE, Phipps EC, Beldavs ZG, Gerding DN, McDonald LC, Gould CV, Lessa FC. 2014. Clostridium difficile infection among children across diverse US geographic locations. Pediatrics 133:651–658. https://doi.org/10.1542/peds.2013-3049.
22. Kociolek LK, Bovee M, Carter D, Ciolino JD, Patel R, O'Donnell A, Rupp AH, Zheng X, Shulman ST, Patel SJ. 17 May 2016. Impact of a healthcare provider educational intervention on frequency of Clostridium difficile polymerase chain reaction testing in children: a segmented regression analysis. J Pediatric Infect Dis Soc https://doi.org/10.1093/jpids/piw027.
23. Fong KS, Fatica C, Hall G, Procop G, Schindler S, Gordon SM, Fraser TG. 2011. Impact of PCR testing for Clostridium difficile on incident rates and potential on public reporting: is the playing field level? Infect Control Hosp Epidemiol 32:932–933. https://doi.org/10.1086/661789.
24. Song L, Zhao M, Duffy DC, Hansen J, Shields K, Wungjiranirun M, Chen X, Xu H, Leffler DA, Sambol SP, Gerding DN, Kelly CP, Pollock NR. 2015. Development and validation of digital ELISAs for ultrasensitive detection and quantification of C. difficile toxins in stool. J Clin Microbiol 53:3204–3212. https://doi.org/10.1128/JCM.01334-15.