Does My Patient Have *Clostridium difficile* Infection?

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Clostridium difficile infection (CDI) seems to be changing—with increasing virulence and incidence, more resistance to metronidazole, and worse outcomes. Accurate diagnosis is critical, but 3 common misconceptions lead to misdiagnosis: *Clostridium difficile* infection is a possibility when the patient has fewer than 3 loose stools per day; the glutamate dehydrogenase test for CDI is sensitive and thus is a good initial test; and repeating an insensitive laboratory test for CDI is useful. These misconceptions can lead to missed diagnoses (for example, when tests with low sensitivity are used) and to false diagnoses (for example, when tests are done in patients who are unlikely to have CDI because they have minimal diarrhea or negative results on recent tests). Diagnoses of CDI will be more accurate if clinicians use tests with a higher sensitivity, reduce the frequency of testing for a single episode of diarrhea, and give more attention to key elements of the patient's history.

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The disease formerly called "*Clostridium difficile*–associated diarrhea" is now called "*C. difficile* infection" (CDI). It is more virulent (1) and more frequent (2) than it used to be, and it responds less often to metronidazole (3). In a time of changing disease characteristics, accurate diagnosis of this infection is critical. Two diagnostic principles have been forgotten and need to be relearned: Clinically significant diarrhea (≥3 stools per day) in a person at risk is a critical criterion for the diagnosis of CDI, and laboratories must detect toxigenic *C. difficile* using either anaerobic stool culture or tests for toxin genes or gene products in the stool (4). The purpose of this article is to explain why these principles are so important.

Which Patients Should I Test?

The standard for suspecting CDI is clinically significant diarrhea, usually defined as 3 or more loose stools per day for at least 1 to 2 days (5). For example, in our recent study of patients suspected of having CDI, one third had fewer than 3 loose stools for 1 day and then had no additional symptoms of CDI during their hospitalization, but two thirds had 3 or more loose stools per day that continued until they were treated if found to have CDI (6). Thus, simply asking about the number of loose stools on the first day of possible CDI readily identifies persons at high risk for having CDI. Other studies have found that 29% to 39% of patients would not need testing if simple similar rules were followed (7, 8). Judicious use of *C. difficile* testing is important because a *C. difficile* colonization state exists and can be common. More than a decade ago, Johnson and Gerding (9) observed that very few patients had *C. difficile* in their stool at the time of hospital admission, but nearly 50% had *C. difficile* in their stool by the end of 4 weeks even though they had no symptoms of CDI. *Clostridium difficile* testing in patients without symptoms of disease is thus analogous to culturing other nonsterile body sites where colonization cannot be distinguished from infection without clinical evidence of disease.

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What Is the Right Test for My Laboratory to Use?

Table 1 describes the types of laboratory tests available for diagnosing CDI (6, 10–13). For at least 10 years, enzyme immunoassays (EIAs) for *C. difficile* toxins in the stool have been the most frequently used diagnostic test for CDI, because they are easy to use and provide same-day results. A decade ago, a study reported toxin A–negative, toxin B–positive strains capable of causing disease (14). These strains were not detected by the most popular EIAs in use at the time, which targeted only toxin A (15), and EIAs targeting both toxins were developed. By 2008, these EIAs accounted for more than 90% of all CDI testing (2, 16). Unfortunately, EIAs are not very sensitive (32% to 73%) (10, 14, 17–19), and alternative tests have been developed.

The glutamate dehydrogenase (GDH) test, or "common antigen" test, was 1 of the first alternatives (20, 21). Initial evaluation of the GDH test found the test to be insensitive, but sensitivity improved when the test was based on EIA instead of latex agglutination (22). Indeed, 2 reports in 2004 suggested that the sensitivity of GDH tests approached 100%. Zheng and colleagues (23) found the sensitivity of 2 GDH tests to be 93% and 94% compared with cytotoxin detection in tissue culture, and Snell and associates (24) reported similar sensitivities using comparable methods. These reports contributed to the incorrect view of many clinicians that GDH has a high sensitivity for detecting *C. difficile*. However, these reports of high sensitivity probably reflected problems in the reference standards. When Zheng and colleagues (23) used anaerobic stool culture as the reference standard, the sensitivity of both GDH tests was only 69%. In Snell and colleagues'

study (24), the culture plates had a low cycloserine concentration, which has been shown to decrease the detection of *C. difficile* (25, 26).

Specificity of the GDH test is too low for it to be used as a standalone assay, so a 2-stage testing concept was investigated to exploit its assumed high sensitivity. This scheme uses GDH testing initially and then retests any specimen with a positive result with a highly specific test, such as EIA or direct stool cytotoxin with tissue culture. The initial GDH test is supposed to identify all of the true-positive results, along with some false-positive results, and the more specific second test is supposed to separate true-positive from false-positive results. Two recent studies have evaluated this approach. Reller and colleagues (11) found that it missed 23% of CDI cases. More recently, an evaluation of a 2-step commercial test found that the GDH component had a sensitivity of 76% (10). Thus, little evidence supports the use of a 2-step scheme with GDH as the initial test for detecting toxigenic *C. difficile*.

Very recently, new quantitative real-time polymerase chain reaction (qPCR) tests have been developed that may provide both high sensitivity and rapid turnaround time. Our study of an in-house qPCR test found a sensitivity of 93.3% and a specificity of 97.4%, versus 73.3% and 97.6% for EIA, 76.7% and 97.1% for direct stool cytotoxin detection with tissue culture, and 100.0% and 95.1% for anaerobic culture for toxigenic *C. difficile* (6). Sloan and colleagues (10) subsequently investigated their qPCR test and found a sensitivity of 86% and a specificity of 97%, versus sensitivities of 32% to 48% and specificities of 85% to 100% for 4 EIAs. Finally, a report by Stamper and colleagues (12) on a new commercial qPCR test found a sensitivity of 84% and a specificity of 98%. In the study, direct stool cytotoxin detection with tissue culture had a sensitivity of 67% and a specificity of 99% (12). Of importance, all these qPCR tests target the toxin B gene, which has recently been shown to be the virulence factor for disease (27). Thus, toxin B seems to be the main, and perhaps sole, virulence factor responsible for CDI, and detecting it must be part of any laboratory diagnosis.

Some observers worry that qPCR tests may be "too sensitive" because they will detect small quantities of colonizing—not disease-causing—*C. difficile* organisms. This concern should apply to all tests for *C. difficile*, but despite this, no one has ever demonstrated that the cases detected by more sensitive tests are more likely than those detected by less sensitive tests to be colonization instead of CDI. Moreover, the greatest protection from this potential problem is to limit *C. difficile* testing to patients with a reasonable probability of having disease, for example, those with

Context

Many clinicians are uncertain how best to evaluate patients with suspected *Clostridium difficile* infection.

Contribution

On the basis of their findings, the authors recommend that only patients with 3 or more loose stools in 1 day be tested. Tests of the stool for a gene that codes for toxin B or its regulators are based on a real-time, quantitative version of polymerase chain reaction (PCR) and provide rapid results. Current enzyme immunoassays of the stool for toxin or glutamate dehydrogenase also provide rapid results. However, quantitative PCR tests are more sensitive. If the initial test result is negative, do not retest. If retesting is necessary, use a different type of test.

Caution

Consensus about these issues has not yet been reached, in part because some developments are so recent.

Implication

Clinicians should reexamine how they evaluate patients with suspected *C. difficile* infection.

—The Editors

Table 1. **Tests Available for Laboratory Confirmation of *Clostridium difficile* Infection***

Test	Description	Sensitivity, %	Specificity, %	Speed of Reports	Cost, $†
EIA	Detects toxin A or toxins A plus B	70–80	>97	Hours	5–17
GDH	Detects a common antigen, not a toxin, of *Clostridium difficile*; immunoassay is preferred over latex agglutination	70–80	<90	Hours	17
qPCR	Detects toxin B or toxin regulator genes; commercial and locally developed tests are available	>90	>97	Hours	7–50
Anaerobic culture for toxigenic *C. difficile*	Detects toxin B	>90	95–97	2 to >3 d	10–22
Direct stool cytotoxin with tissue culture	Detects toxin B	70–80	>97	2 to >3 d	7–13

EIA = enzyme immunoassay; GDH = glutamate dehydrogenase; qPCR = quantitative real-time polymerase chain reaction.
* Adapted from references 6 and 10–13.
† Range of manufacturer's suggested retail prices for 2007–2008 (6, 12).

Table 2. **Model of Results for Toxigenic *Clostridium difficile* Detection When Testing Is Repeated***

Test Sequence	EIA						qPCR					
	Tested, *n*	True Positive, *n*	PPV	False Positive, *n*	Undetected Disease, *n*	Remaining Negative Results, *n*	Tested, *n*	True Positive, *n*	PPV	False Positive, *n*	Undetected Disease, *n*	Remaining Negative Results, *n*
First	1000	73	0.75	24	27	903	1000	93	0.78	26	7	881
Second	903	18	0.45	22	9	863	881	7	0.23	23	0	851
Third	863	7	0.25	21	2	835	–	–		–	–	
Fourth	835	1	0.05	20	1	814	–	–		–	–	
Fifth	814	1	0.05	20	0	793	–	–		–	–	
Total		100†		107				100‡		49		

EIA = enzyme immunoassay; PPV = positive predictive value; qPCR = quantitative real-time polymerase chain reaction.
* In this model, there are 1000 tested participants and *C. difficile* prevalence in the test population is 10%. Patients with negative results have tests repeated sufficiently to ensure that all true-positive results are captured. Assumptions for EIA: sensitivity = 73.3%; specificity = 97.6%; and test performance does not change when repeated (6). Assumptions for qPCR: sensitivity = 93.3%; specificity = 97.4%; and test performance does not change when repeated (6).
† Overall PPV = 0.48.
‡ Overall PPV = 0.67.

3 or more loose stools per day for 1 to 2 days. Taken together, these data suggest that qPCR to detect the gene for toxin B, when used in an appropriate clinical setting, is the most sensitive and specific test available for demonstrating the presence of toxigenic *C. difficile* in the stool of patients with possible CDI, and results from this test can be available the same day the specimen is collected.

When Should I Order Another Stool Test (or, When Is 3 Times *Not* a Charm)?

Many clinicians routinely perform multiple—usually 3—EIAs for *C. difficile* in rapid succession to overcome the known insensitivity of the test, and an early report found a 12% increase in sensitivity when the EIA was repeated (28). Unfortunately, this strategy overlooks 2 critical issues. The stool of a patient with a false-negative test result may, for example, contain something that will continue to cause test results to be falsely negative on subsequent testing, although no data about this possibility are available. The second critical issue is that the probability of disease decreases with each negative test result, which means that the test's positive predictive value is lower when the second and third tests are done. **Table 2** illustrates this effect for EIA and qPCR. The positive predictive value for the second test with either testing strategy is less than 50%. This effect explains what occurred in 1 study of patients who had at least 3 EIA tests performed within 7 days when the first test result was negative (29). In these 20 patients, 10 had negative EIA test results before and after a positive result, and in all but 3 patients, more results were negative than positive, making it impossible to know which positive test results were true-positive results. Also, no patient had a negative qPCR result followed by a positive qPCR result, which illustrates the value of using a test with a high sensitivity.

Conclusion

We believe that accurate diagnosis of CDI is critical. The diagnosis should incorporate information about the patient's clinical presentation (4–9) and results from a diagnostic test that identifies toxin B in the stool or pseudomembranes in the colon. A prudent approach is to use the most sensitive rapid test first and not to repeat that test for 7 to 14 days. If another test on stool from the same patient is required, the repeated test should be of a different type.

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