



Clostridioides (Clostridium) difficile Testing

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SYNOPSIS AND RELEVANCE

There are several tests available for the diagnosis of *Clostridioides* (formerly *Clostridium*) *difficile* infection. When testing stool samples for the presence of *C. difficile*, it is important that appropriate pre-analytic testing criteria are applied along with timely reporting of results to:

1. Ensure patients with *C. difficile* infection are identified and treated
2. Avoid nosocomial transmission of *C. difficile* through prompt implementation of infection prevention precautions and effective cleaning of rooms after patient discharge
3. Avoid unnecessary testing of patients who lack signs and symptoms of *C. difficile* infection
4. Optimize institutional *C. difficile* incidence rates

OBJECTIVES

1. To understand the relationship between diagnostic test sensitivity/specificity and appropriateness of pre-analytic criteria.
2. To understand the clinical and laboratory technical issues that impact tests used to detect *C. difficile* infection.
3. To create a plan to collaborate with clinical colleagues and nurses to facilitate the appropriate utilization of laboratory tests used to diagnose *C. difficile* infection.

BACKGROUND

C. difficile is a diarrheagenic, gram-positive, spore-forming bacterium that is easily transmitted between patients and difficult to eradicate from the healthcare environment. It is therefore one of the most common causes of hospital-acquired infections.¹ The virulence of the organism is mediated by two toxins: toxin A (enterotoxin) and toxin B (cytotoxin).

Patients with *C. difficile* infection (CDI) can have symptoms ranging from foul smelling, watery diarrhea with abdominal pain to severe fulminant enterocolitis.² These symptoms are often accompanied by an elevated white blood cell count and fever.³ Paradoxically, this organism can also colonize the gastrointestinal tract without causing disease.⁴ Colonization, also known as carriage, does not need to be treated. Therefore, it is extremely important to test only the appropriate patients and interpret the results in the clinical context.^{5,6} For the best clinical diagnostic performance, clinicians and laboratories should only test diarrheal stool in patients who have had 3 or more loose stools in the past 24 hours, should not repeat tests in positive cases within certain time intervals determined by your institution, and should not perform test of cure. Nursing documentation of stool frequency, volume, and consistency, or at least the presence of diarrhea, and documentation of absence of recent laxative use is important to assure appropriate testing. Finally, laboratories can consider enforcing specimen acceptance and rejection criteria such as accepting only specimens that take the shape of their container (ie, unformed specimens).

There are numerous assays and algorithms used to detect *C. difficile*.^{7,8}

- **Cell Culture Cytotoxicity Neutralization Assay (CCCNA)**
The cytotoxicity assay, a gold standard test for detecting *C. difficile* toxin in a fecal sample,⁹ is labor-intensive, and requires 18 to 48 hours incubation time before a final reading can be made.¹⁰
- **Toxigenic culture**
Toxigenic culture, like the cytotoxicity assay, requires significant time (2-5 days) and labor; therefore, it is generally regarded as a reference method rather than a primary diagnostic test. It involves the recovery of *C. difficile* by anaerobic culture paired with a method to assess toxin production.

- Polymerase chain reaction (PCR)
Nucleic acid amplification assays have the highest sensitivity and can be performed easily and quickly. These assays may be used to rule out CDI, but cannot distinguish colonization from infection. It is, therefore, imperative to only test patients with risk factors for CDI (unexplained new onset diarrhea; 3 or more unformed stools/day; no recent laxative use). The 2018 Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) guideline recommends use of a stand-alone PCR test only if clinicians have agreed to limit testing to patients meeting the pre-analytic criteria for CDI.⁶
- Enzyme immunoassay (EIA)
The low sensitivity of toxin EIA makes it an unreliable test to rule out disease. Missing significant CDI led to the development of molecular methods available since 2009. Some institutions opt to screen with a multi-step algorithm that includes the detection of glutamate dehydrogenase (GDH) antigen specific to *C. difficile* followed by toxins A and B EIA to detect toxin-producing strains. If the EIA is negative for both of those targets (GDH and toxin), the test result is considered negative; if both are positive, the test is deemed positive; if only the screening GDH EIA is positive, then a reflex PCR can be employed, with the result of the PCR determining the test algorithm result. This algorithm delays the final test result and has lower sensitivity than PCR alone (GDH is not 100% sensitive).

Theoretically, the presence of toxin detectable by a less sensitive EIA makes infection more likely than colonization. Hence, the IDSA/SHEA guideline suggests that if clinicians in an institution have not agreed to limit PCR testing to patients with unexplained diarrhea and no recent laxative use, then a multi-step algorithm that includes a toxin EIA is recommended.⁶ Use of an algorithm may be less expensive in terms of laboratory costs, but downstream costs (ie, slower time to discontinuation of isolation when a test is negative; increased transmission because of the lower sensitivity) may be higher.

Following release of the updated IDSA guideline, the National Healthcare Safety Network (NHSN) that monitors *C. difficile* Laboratory Identified (LabID) events in the United States adjusted guidance to focus reporting on only the final test in a multi-step algorithm.¹¹ This has led many laboratories to adopt a reflex test algorithm with toxin EIA as the last step for PCR positive specimens.¹² Results are reported with a comment noting that EIA is less sensitive than PCR and clinical correlation is required to determine if PCR positive, EIA negative results represent infection or colonization.

- PCR-Based Panels
C. difficile PCR is also available as one of several analytes on a panel that detects gastrointestinal pathogens. This poses interesting dilemmas when determining which patients to test because a patient may not have a history consistent with CDI, yet this organism is tested as part of the panel; in such circumstances, an existing positive result may be difficult to ignore. This dilemma is especially apparent in pediatric patients, in whom carriage rates are substantial and testing for numerous other pathogens via a panel is warranted. Some laboratories using large gastrointestinal panels have chosen to block or suppress panel results for *C. difficile* and instead use only stand-alone *C. difficile* tests. This can result in CDI being missed. Another approach is to add a toxin EIA as the final test when a *C. difficile* molecular result is positive and add a comment noting the low sensitivity of EIA and that clinical correlation is required to determine if PCR positive, EIA negative results represent infection or colonization.

INSIGHTS

1. *C. difficile* can cause severe disease in some patients but other patients may carry it asymptotically in their stool. Limiting testing to specific situations (eg, testing only after three loose stools within 24 hours) is important for correlating test results to patient disease.
2. Toxin EIAs lack sensitivity and cannot be considered reliable in ruling out disease. A positive *C. difficile* test, particularly a nucleic acid amplification test, does not always mean that the patient has *C. difficile* disease and therefore the patient does not always need to be treated. Clinical correlation is essential.
3. Strict testing criteria should be in place to assure that only appropriate patients are being tested for *C. difficile*.
 - a. Routine testing should be avoided in patients who are less than 2 years of age.
 - b. Patients should not be on nasogastric feeds (if they are on tube feeds, the protocol should not have been changed within the past 24 hours).
 - c. Patients should not be on or have recently been on laxatives at the time of testing for *C. difficile*.
4. Certain criteria should be in place to assure that only appropriate patients are tested:
 - a. Do not test formed stool.
 - b. Do not perform *C. difficile* tests, particularly PCR, as a test of cure.
 - c. Establish limitations on time intervals for repeat testing following a positive test (eg, 7 days).

INTERVENTIONS

1. Develop and enforce a procedure that requires review of the characteristics of the stool specimen submitted for testing, with rejection of certain specimens if necessary. Involve nursing in the documentation of stool characteristics, frequency, and volume. Nursing education emphasizing the importance of accurate documentation is essential.
2. Limit orders based on timing by one of the following methods:
 - a. Create a best practice alert, such as a “pop-up” or soft stop, whenever a second *C. difficile* test is ordered within a designated time period (eg, 7 days per IDSA/SHEA guidelines). The clinician may override this type of alert at the point of computer order entry when appropriate.
 - b. Create a hold (also referred to as a hard-stop, which is an intervention that requires permission to override) on the order whenever a second *C. difficile* study is ordered within a designated time period (eg, 7 days per IDSA/SHEA guidelines). This type of intervention cannot be overridden at the point of order entry, so information must be provided regarding how the test can be obtained, if there is a medically valid reason for repeating the study. An example of a medically valid reason would be the development of diarrhea in a previously negative patient who initiated a new antimicrobial treatment.
3. Build questions into the electronic order entry screen for physicians, or include questions in a nursing protocol if nurses can order *C. difficile* tests, such as:
 - a. Has the patient been on laxatives in the past 48 hours?
 - b. Has the patient had at least 3 loose stools in the past 24 hours? (Ideally, a link to a Bristol Stool Chart would be readily available).
 - c. Has the patient been on nasogastric tube feeds, or had tube feeds changed or modified, in the past 24 hours?
4. Limit tests of cure by:
 - a. Creating a best practice alert, such as a “pop-up” or soft stop, whenever a repeat *C. difficile* test is ordered on a previously positive patient for a test of cure. The clinician may override the alert at the point of computer order entry. There is no clinical value in repeat *C. difficile* testing to establish cure.⁸
 - b. Or, creating a hold (also referred to as a hard-stop, which is an intervention that requires permission to override) on the order whenever a repeat *C. difficile* test is ordered on a previously positive patient for a test of cure. This type of intervention cannot be overridden at the point of order entry, so information must be provided regarding how the test can be obtained, if there is a medically valid reason for repeating the studies. An example of a medically valid reason would be concern over a mislabeled specimen.
5. Review clinical ordering patterns. If a relatively high number of repeat *C. difficile* studies on previously positive patients and/or repeat test requests are identified among specific clinicians or locations, then a focused examination to uncover potential utilization problems may be helpful. Alternatively, selective feedback to clinicians about ordering practices relative to peers (eg, physician score cards) may also lead to improved utilization.
6. Collaboratively work with infection prevention and other stakeholders to determine the most appropriate approach to diagnosis of CDI for your institution. Review standing orders, panels, reflex testing workflows, and diagnostic aids that contain *C. difficile* tests to assure that they are appropriately designed and used. Provide ordering clinicians with educational information about this method.

INTERVENTION ANALYSIS

To optimize the utilization of tests commonly used to detect *C. difficile*, monitor the number of rejected specimens (non-diarrheal stool specimens). Also, monitor the number of orders received within a certain time frame of a previous test. Determine how many patients being tested for *C. difficile* are on laxatives or tube feeds. Finally, review the number of orders placed on patients less than (<) 1-year-old. Consider which of the above interventions are possible at your institution. After interventions have been implemented, determine the impact on test ordering practices. (Appendix A).

APPENDIX A: DETERMINATION OF INTERVENTION IMPACT ON *C. DIFFICILE* TESTING

Collect data in the table below for a 1-month period before and after implementing interventions.

Laboratory Test Volume Outcomes and Opportunities				
Description	Pre-Intervention	Post Intervention	Volume Change	Percent Change (%)
Number of total <i>C. difficile</i> test orders	A1	A2	A1 - A2 = A3	A3/A1 x 100% = A4%
Number of repeat <i>C. difficile</i> test orders (eg, two or more orders within one 7-day period)	B1	B2	B1 - B2 = B3	B3/B1 x 100% = B4%

Laboratory Test Volume Outcomes and Opportunities (con't)				
Description	Pre-Intervention	Post Intervention	Volume Change	Percent Change (%)
Number of <i>C. difficile</i> tests ordered on non-diarrheal stools	C1	C2	$C1 - C2 = C3$	$C3/C1 \times 100\% = C4\%$
Number of <i>C. difficile</i> tests performed on patients who have received laxatives within the previous 48 hours	D1	D2	$D1 - D2 = D3$	$D3/D1 \times 100\% = D4\%$
Number of <i>C. difficile</i> tests performed on patients < 1-year-old	E1	E2	$E1 - E2 = E3$	$E3/E1 \times 100\% = E4\%$

QUESTIONS AND ANSWERS

QUESTION 1

OBJECTIVE

To understand the relationship between diagnostic test sensitivity/specificity and appropriateness of patient selection.

What diagnostic assay or combination of assays has high sensitivity for detection of *C. difficile* in fecal specimens and can be performed easily and quickly?

- A. Toxigenic culture
- B. Polymerase chain reaction
- C. An algorithm starting with a combination GDH and toxin EIA and proceeding to a PCR if GDH+/toxin-
- D. An algorithm starting with PCR and reflexing to toxigenic culture if negative

The correct answer is B. Polymerase chain reaction (PCR) for *C. difficile* toxin genes is fast and relatively easy to perform; therefore, it is commonly used in clinical laboratories. However, appropriate patient selection is imperative as the high sensitivity of this test may detect patients with colonization.

A is incorrect. Toxigenic culture requires significant time and labor.

C is incorrect. Although this algorithm is used in some clinical laboratories, it has lower sensitivity compared with PCR.

D is incorrect. This algorithm is not used in clinical laboratories. PCR has high sensitivity and no additional testing is needed if negative.

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Burnham CA, Carroll KC. Diagnosis of *Clostridium difficile* infection: an ongoing conundrum for clinicians and for clinical laboratories. *Clinical Microbiology Reviews*. 2013;26(3):604-630. doi:10.1128/CMR.00016-13

QUESTION 2

OBJECTIVE

To understand the clinical and laboratory technical issues that impact tests used to detect *C. difficile* infection.

Why is it important to only test patients with signs and symptoms of *C. difficile* infection (CDI) for *C. difficile*?

- A. Commonly used diagnostic tests such as PCR are very sensitive; therefore, they can detect low quantities of organisms which may not be causing disease.
- B. Patients who were previously infected with *C. difficile* can have prolonged shedding of the organism; therefore, "test of cure" is not recommended once the patient is asymptomatic.
- C. The presence of *C. difficile* in a patient's gastrointestinal tract can cause severe disease in some patients but may be asymptomatic in other patients.
- D. All of the above

The correct answer is D. All of the above describe reasons why only patients with signs and symptoms of *C. difficile* infection (CDI) should be tested.

A is incorrect. Although this is a true statement, other answers are also correct, making D the best answer.

B is incorrect. Although this is a true statement, other answers are also correct, making D the best answer.

C is incorrect. Although this is a true statement, other answers are also correct, making D the best answer.

REFERENCE

Cohen SH, Gerding DN, Johnson S, et al. 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*. 2010;31(5):431-455. doi:10.1086/651706

QUESTION 3

OBJECTIVE

To create a plan to collaborate with clinical colleagues and nurses to facilitate the appropriate utilization of laboratory tests used to diagnose *C. difficile* infection.

Testing for *C. difficile* should not be routinely performed on which of these patients?

- A. Patients on laxatives
- B. Patients less than 2 years old
- C. Patients with formed stools
- D. Asymptomatic patients needing placement in a long-term rehabilitation facility
- E. All of the above

The correct answer is E. All of the above are patients who should NOT be tested for *C. difficile*. Testing should only be performed on patients with signs and symptoms of *C. difficile* infection (CDI).

A is incorrect. Patients with true *C. difficile* disease have diarrhea and will not need laxatives in order to have a bowel movement. However, this is not the best answer.

B is incorrect. Upwards of 50% of patients under 1 year of age can be carriers of *C. difficile*, therefore, they should not be routinely tested. However, this is not the best answer.

C is incorrect. Patients with formed stool are unlikely to have *C. difficile* disease. However, this is not the best answer.

D is incorrect. Patients can have asymptomatic shedding for several months following infection and therefore “test of cure” is not recommended. However, this is not the best answer.

REFERENCE

Cohen SH, Gerding DN, Johnson S, et al. 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*. 2010;31(5):431-455. doi:10.1086/651706

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