



Clostridioides (Clostridium) difficile Testing

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

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).
- **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).

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