

ID Learning Unit: Understanding and Interpreting Testing for *Clostridium difficile*

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Understanding and interpreting the molecular tests for *Clostridium difficile* is challenging because there are several different types of assays and most laboratories combine multiple tests in order to assess for presence of disease. This learning unit demonstrates the basic principles of each test along with its strengths and weaknesses, and illustrates how the tests are used in clinical practice.

Keywords. *Clostridium difficile*; laboratory testing.

A 68-year-old woman received a dose of clindamycin prior to a routine dental procedure. The next day she developed watery diarrhea. Testing for *Clostridium difficile* is reported as follows: "Antigen" positive, "Toxin" negative, "PCR" pending.

How do you interpret these results, and why are there so many tests?

If you feel confused when trying to interpret tests for antibiotic-associated *Clostridium difficile*, rest assured you are not alone. There are multiple different assays, and most microbiology laboratories utilize a combination of these tests to optimize for sensitivity and specificity, control cost, and ensure a rapid turnaround time to avoid delays in therapy. This learning unit is designed to help (1) you understand the value and limitations of the various tests and (2) demonstrate how they are used together in clinical practice.

An essential concept to understand is that not all *C difficile* bacteria cause the prototypical diarrheal illness. If all *C difficile*

resulted in disease, testing would be easy, and we would simply pick the test that performs best at detecting the presence of bacteria in the stool or the colon! Whether the bacteria causes clinical symptoms depends on both bacterial and host factors.

After adhering to the colonic mucosa, pathogenic *C difficile* releases 2 different toxins, toxin A and toxin B, that are directly cytotoxic to the colonic epithelial cells and cause indirect destruction by activating an inflammatory response in the host. However, not all strains of *C difficile* are equipped with the gene that produces toxin, and bacteria that do not produce toxin are not pathogenic [1]. Furthermore, even toxigenic strains of *C difficile* can live harmlessly in the colon without producing toxin. A complex balance of host factors, including immune function, exposure to medications such as antibiotics or immunosuppressants, and the host microbiome, mediates whether a toxigenic organism actively produces toxin [2]. Understanding this balance is particularly important because the prevalence of asymptomatic carriers—patients who have toxigenic *C difficile* but no toxin production—is rising, especially among patients in healthcare settings. Prospective screening studies have shown that up to 12%–14% [3, 4] of patients admitted to the hospital are colonized with toxigenic strains of *C difficile*, and that number may be even higher in long-term care facilities [5].

So when we test for *C difficile* what are we actually testing for? The presence of any bacteria, the presence of toxigenic bacteria, the presence of the toxin itself, or the activity of toxin on human cells? The answer is that it depends on the test you are sending. The four most commonly used tests are described below and summarized in Table 1.

Test: Enzyme-linked immunosorbent assay (ELISA) for the glutamate dehydrogenase (GDH) antigen

What it tests for: The presence of *C difficile* organisms

Commonly known as the antigen test, this test uses antibodies to test for presence of the GDH enzyme, a protein preserved in all *C difficile* bacteria. It is an excellent screening test because it has sensitivity greater than 90%, a rapid turnaround time of 15–45 minutes, and it is inexpensive. However, this assay does not assess for toxin production, and because the GDH is present in all bacterial cells, it cannot even distinguish between toxigenic and non-toxigenic strains of the bacteria, so its specificity for active disease is poor [6].

Test: Polymerase chain reaction

What it tests for: The presence of toxigenic *C difficile* organisms

Polymerase chain reaction (PCR) is a highly sensitive laboratory technique that uses DNA primers to amplify copies of a

Received and accepted 16 March 2014.

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Open Forum Infectious Diseases

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Table 1. Summary of laboratory tests for *C difficile*

Laboratory Test	Sensitivity	Specificity	Time to results	Comment
ELISA for Antigen	High	Low	Rapid	Cannot distinguish between toxigenic and non-toxigenic strains
ELISA for Toxin	Low	High	Rapid	Easy to perform; poor sensitivity
PCR for toxigenic genes	High	High	Rapid	Cannot distinguish between active infection and asymptomatic carriage; more expensive than ELISA
Cytotoxin Assay	High	High	Slow (24-48hrs)	Gold standard; requires tissue culture facility

targeted gene in a test sample. *Clostridium difficile* PCR is designed to amplify 2 different genes that are specific to toxigenic strains of *C difficile*: *tcdB*, which encodes for toxin B, and *tcdC*, which encodes for a toxin regulatory pathway. *Clostridium difficile* bacteria that do not produce toxin do not carry this gene, so it is specific for toxigenic strains of *C difficile* [7, 8].

A highly sensitive and specific test sounds ideal, so why don't we just use this test alone? First, it is relatively expensive. Given the frequency with which we test for *C difficile*, ELISA is a more cost-effective screening tool. Second, although it can distinguish between toxigenic and non-toxigenic strains of the bacteria, it still does not test for active toxin production, so it picks up asymptomatic carriers of disease. Moreover, in patients who have active *C difficile* and complete an adequate course of antibiotic therapy, the PCR often remains positive, so it is a challenging test to interpret in patients who have had *C difficile* in the past.

Test: ELISA for toxin

What it tests for: The presence of toxin

Using the same technique as the antigen test, this assay uses antibodies to detect the presence of *C difficile* toxin A or toxin B. The specificity of this test is nearly perfect, and it is therefore an excellent test to confirm active disease. Similar to the antigen test, this test can be performed quickly (15–45 minutes) and is inexpensive. The pitfall of the toxin assay is its low sensitivity (75%), so it results in a high rate of false negatives [9].

Test: Cytotoxin neutralization assay

What it tests for: Toxin activity

A functional assay that tests for cytopathic effect on human tissue cells, this is our gold standard laboratory test. The specimen is prepared by centrifuging liquid stool samples, harvesting the supernatant, and then inoculating different dilutions onto a monolayer of human foreskin cells in cell culture. The sample is then monitored at different time intervals for cytotoxicity. If cell disruption is observed, a *C difficile* antitoxin is added to a second cell culture with the patient sample and monitored for absence of cytotoxicity. The combination, cytotoxicity, and abrogation by antitoxin specific to *C difficile*, is diagnostic.

The unique value of this test is that it simulates the clinically important outcome: is there a toxic effect on human cells? It is

highly specific and sensitive and, historically, was the preferred test for most laboratories. The downside of this test is that it takes a long time—clinicians may have to wait up to 48 hours before results are reported (or even longer over a weekend), which leads to delays in therapy and hospital workflow. Furthermore, performing and interpreting the test is work intensive, and it relies on the technique of laboratory technicians, introducing an element of user variability [10]. With the addition of cheaper and faster tests, the cytotoxin assay has fallen out of favor, but there is still a role for its use in complicated cases when alternative tests are confusing or contradictory. In any situation in which a new testing modality is being validated, cytotoxicity assay should be used as the gold standard.

So how are these tests actually used in clinical practice? First, you should be careful to order *C difficile* testing only for patients with appropriate clinical history, which should include loose or poorly formed stools and/or diarrhea. Because each single test has individual pitfalls, most laboratories combine multiple tests to optimize sensitivity and specificity as well as ensure that results are delivered to clinicians in a timely manner. Below is a typical algorithm that combines several tests.

Step 1: ELISA for GDH antigen and ELISA for toxin (Figure 1) [11]

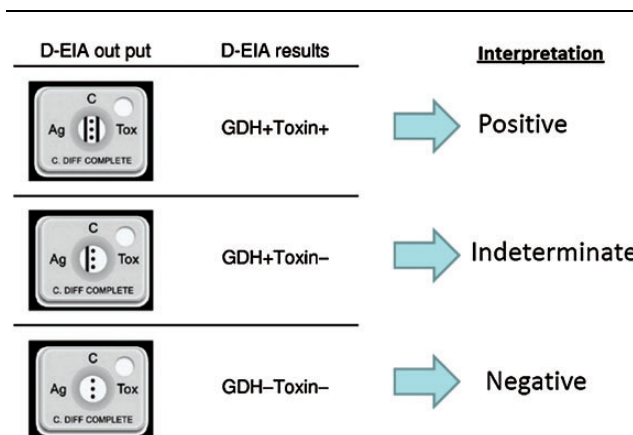


Figure 1. Depiction of combined Antigen and Toxin ELISA assay with test interpretation.

When used together, these tests provide a powerful screening test. They are inexpensive, rapid, and when their results are concordant they have both a high sensitivity and high specificity. If both tests are positive, the assay is reported as positive and no further tests are required. If both tests are negative, the assay is reported as negative and no further tests are required. Here is where it gets a bit complicated: if the antigen is positive, but the toxin is negative, the assay is reported out as indeterminate. There are several possible explanations for an indeterminate result:

1. The patient has non-toxigenic *C difficile*, and the toxin test was a true negative.
2. The patient is an asymptomatic carrier of toxigenic *C difficile*, and the toxin test was a true negative, but does not have active disease.
3. The patient has active toxigenic *C difficile* and the toxin test was a false negative.

In this case, we utilize a third test to act as a tie breaker.

Step 2: PCR

If the PCR is positive, we can determine with certainty that the patient has toxigenic *C difficile*, and the final interpretation is positive. It is important to recall that the PCR still does not distinguish between active disease and asymptomatic carriage. This point of distinction remains a challenge in the diagnosis of *C difficile*, and it serves as an important reminder that testing must be interpreted in the clinical context. If you suspect that the test is identifying asymptomatic carriage in your patient, it might be helpful to ask your laboratory to dig up the old cytotoxin assay to test for disease activity.

If you are still scratching your head, you may be tempted to abandon laboratory testing altogether and enlist the help of Cliff, the adorable 2-year-old beagle, who has been trained to sniff out toxigenic strains of *C difficile* [12]. Unfortunately, his effectiveness runs into the same pitfalls as the PCR—he cannot distinguish between active *C difficile* and asymptomatic colonization. There is not yet enough data for us to endorse the mythical combination of pet therapy and 4-legged *C difficile* testing as a scalable, cost-effective technique.

Note

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Kelly CP, Pothoulakis C, Lamont JT. *Clostridium difficile* colitis. N Engl J Med **1994**; 330:257–62.
2. Britton RA, Young VB. Role of the intestinal microbiota in resistance to colonization by *Clostridium difficile*. Gastroenterology **2014**; pii:S0016-5085(14)00150-4.
3. Guerrero DM, Becker JC, Eckstein EC, et al. Asymptomatic carriage of toxigenic *Clostridium difficile* by hospitalized patients. J Hosp Infect **2013**; 85:155–8.
4. Kyne L, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. N Engl J Med **2000**; 342:390–7.
5. Simor AE, Bradley SF, Straughnsbaugh LJ, et al. *Clostridium difficile* in long-term-care facilities for the elderly: SHEA Position Paper. Infect Control Hosp Epidemiol **2002**; 11:696–703.
6. Wilkins TD, Lyerly DM. *Clostridium difficile* testing: after 20 years, still challenging. J Clin Microbiol **2003**; 41:531–4.
7. Barbut F, Braun M, Burghoffer B, et al. Rapid detection of toxigenic strains of *Clostridium difficile* in diarrheal stools by real-time PCR. J Clin Microbiol **2009**; 47:1276–7.
8. Stamper PD, Alcabasa R, Aird D, et al. Comparison of a commercial real-time PCR assay for *tcdB* detection to a cell culture cytotoxicity assay and toxigenic culture for direct detection of toxin-producing *Clostridium difficile* in clinical samples. J Clin Microbiol **2009**; 47:373–8.
9. Burnham CA, Carroll KC. Diagnosis of *Clostridium difficile* infection: an ongoing conundrum for clinicians and for clinical laboratories. Clin Microbiol Rev **2013**; 26:604–30.
10. Landry ML, Ferguson D, Topal J. Comparison of Simplexa Universal Direct PCR with cytotoxicity assay for diagnosis of *Clostridium difficile* infection: performance, cost, and correlation with disease. J Clin Microbiol **2014**; 52:275–80.
11. Figure adapted from: Quinn CD, Sefers SE, Babiker W, He Y, Alcabasa R, Stratton CW, Carroll KC, Tang YW. *C. Diff* Quik Chek Complete Enzyme Immunoassay Provides a Reliable First-Line Method for Detection of *Clostridium difficile* in stool specimens. J Clin Microbiol **2010**; 48:603–5.
12. Bomers MK, van Agtmaale MA, Luik H, et al. Using a dog's superior olfactory sensitivity to identify *Clostridium difficile* in stools and patients: proof of principle study. BMJ **2012**; 345:e7396.