Evidence-Based Commentary: Testing for *Clostridioides difficile* Infection

Sahil Khanna[1]

[1] Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota, United States

J Gastrointest Infect 2023;13:95–98.

Address for correspondence Sahil Khanna, MBBS, MS, FACG, AGAF, Division of Gastroenterology and Hepatology, Mayo Clinic, 200 First St SW, Rochester, MN 55905, United States (e-mail: Khanna.sahil@mayo.edu).

What Is *Clostridioides difficile* Infection, and Why Is It a Significant Healthcare Concern?

Clostridioides difficile infection (CDI) is a bacterial infection of the large intestine caused by the gram-positive, spore-forming bacterium, *C. difficile*. It is a significant healthcare concern due to low quality of life, an increasing incidence, high morbidity, recurrence rates, and economic burden.[1,2] Furthermore, CDI is a leading cause of healthcare infection, affecting hospitalized and recently discharged patients. Its contagious nature poses a risk to healthcare facilities. *C. difficile* infection is associated with various risk factors, the most prominent being recent antibiotic exposure, particularly broad-spectrum antibiotics, which disrupt the normal gut microbiota and allow *C. difficile* to proliferate.[3] Other risk factors include advanced age, longer length of stay, chemotherapy, immunosuppression, and gastrointestinal surgery. *C. difficile* infection presents with diarrhea, abdominal pain, and fever, and in severe cases, it can lead to pseudomembranous colitis, toxic megacolon, or even sepsis. Given the increasing prevalence and severity of CDI, timely and accurate diagnosis is crucial to guide appropriate therapy, reduce transmission, and improve patient outcomes.

What Are the Available Diagnostic Methods for *C. difficile* Infection, and How Do they Compare in Terms of Accuracy, Sensitivity, and Specificity?

There is no perfect test for the diagnosis of CDI and should be performed and interpreted in context of symptoms. Patients without risk factors or symptoms of CDI should not be tested. Several diagnostic methods are available for CDI, with varied sensitivity, specificity, advantages, and limitations (►**Table 1**).[4] These include nucleic acid amplification tests (NAATs), toxin detection tests, and culture-based assays. Culture-based methods are the gold standard, allowing for strain typing and antimicrobial susceptibility testing. They are slow and have lower sensitivity due to the requirement for viable *C. difficile* organisms. Cell culture cytotoxicity neutralization assay is not used in clinical practice as it is time consuming, cumbersome and can lead to delayed results. It is generally used in research settings. While pseudomembranes have high sensitivity and specificity for CDI, endoscopy is invasive. If stool tests do not demonstrate CDI, and there is a high suspicion, endoscopic evaluation for pseudomembranes can be performed. If pseudomembranes are seen on endoscopic evaluation, treatment for CDI can be initiated in the appropriate clinical context.

Polymerase chain reaction (PCR) is a form of NAAT, offers high sensitivity and specificity, and is preferred due to speed and accuracy in detecting the presence of *C. difficile* DNA. However, it does not detect presence of toxin, and therefore do not distinguish between colonization and active infection. This may lead to over diagnosis, especially in the absence of symptoms or symptoms explained by other causes.

Toxin detection tests, such as an enzyme immunoassays (EIA) for the presence of toxin A & / B, are used to detect *C. difficile* toxins in stool samples.[5] These are cost-effective and provide rapid results but have a lower sensitivity leading to missed cases.[4] This low sensitivity is related to the performance of the assay (a high lower limit of detection) or toxin degradation due to stool handling and delays in performing the test. In order to achieve the best diagnostic accuracy, a two-step approach is often recommended, starting with a sensitive test (but not specific) like an antigen

received
October 16, 2023
first decision
October 18, 2023
accepted
October 19, 2023

DOI https://doi.org/10.1055/s-0043-1777090.
ISSN 2277-5862.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

Table 1 Characteristics of diagnostic tests for *Clostridioides difficile* infection. Reprinted with permission[4]

Test method	Target	Characteristic	Sensitivity	Specificity
EIA	GDH	Insufficient for diagnosis alone; needs confirmation with toxin testing; common first diagnostic test	0.88–0.92 (0.6–1.0)	0.89–0.93 (0.75–1.0)
EIA	Toxin A or toxin B	Variable accuracy; tests toxin production; used as a first step or to confirm a positive GDH test	0.73–0.87 (0.32–0.99)	0.97–0.98 (0.65–1.0)
NAAT or PCR	*tcdB or tcdC* gene	Widely available but more expensive; can be used when GDH and toxin EIA are discordant; NAAT alone may increase detection of asymptomatic colonizers	0.87–0.92 (0.84–1.0)	0.94–0.97 (0.94–1.0)
Multistep algorithms	GDH, toxin A or toxin B, *tcdB* or *tcdC* gene	High accuracy; may help distinguish true CDI from C *difficile* colonization; when results are discordant (i.e., GDH positive and toxin negative), NAAT testing can confirm the correct diagnosis	Range, 0.68–1.0	Range, 0.92–1.0

Abbreviations: EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; NAAT, nucleic acid amplification tests; PCR, polymerase chain reaction.

(glutamate dehydrogenase/GDH) and confirming positive results with a toxin assay (►**Fig. 1**).[6]

What Are the Advantages and Limitations of Nucleic Acid Amplification Tests (NAATs) in Diagnosing *C. difficile* Infection?

PCR has revolutionized the diagnosis of CDI, offering advantages, including high sensitivity and specificity, rapid results, and the ability to detect the presence of *C. difficile* DNA in stool samples.[4] Despite limitations, NAATs have become increasingly popular in clinical practice due to their accuracy and speed. Advantages include their ability to detect cases missed by toxin-based tests, making them suitable for high-risk patient populations.[7] Rapid turnaround times aid in timely clinical decision-making, which is crucial for infection control and management. The main limitation of NAATs is their inability to distinguish between colonization and active infection. They detect the presence of *C. difficile* DNA, but not necessarily the production of toxins causing symptoms. This leads to the potential overdiagnosis of CDI, particularly in patients without clinical symptoms. Overreliance on NAATs may contribute to unnecessary treatment and increased healthcare costs. Therefore, it is important for clinicians to interpret NAAT results in the context of clinical symptoms and risk factors. In summary, NAATs are highly sensitive and specific tools for CDI diagnosis, but clinical judgment is crucial to avoid overtreatment.[6,7]

What Are the Advantages and Limitations of Toxin Detection Tests in Diagnosing *C. difficile* Infection?

Toxin detection tests, such as EIAs, have been used to diagnose CDI way before PCR-based assays. Tests for detection of toxin by EIA have advantages and limitations that clinicians should consider. Advantages include their low cost and relatively rapid turnaround time. These are widely available and can detect the presence of toxins produced by *C. difficile* in stool samples, providing an indirect measure of toxin production. However, toxin detection tests have limitations. They are less sensitive compared to NAATs and may miss cases of CDI due to low sensitivity.[6,7]

While toxin tests have been used historically, they are increasingly being complemented or replaced by NAATs, which offer higher sensitivity. Clinicians should consider the specific clinical context, patient population, and local epidemiology when choosing a diagnostic approach. In some cases, a two-step method involving initial GDH testing followed by toxin confirmation may provide the best balance of sensitivity and specificity (►**Fig. 1**).[6]

What Are the Current Guidelines or Recommendations for *C. difficile* Testing?

Clinical societies including the American College of Gastroenterology, Infectious Diseases Society of America, and European Society of Clinical Microbiology and Infectious Diseases have established guidelines and recommendations for CDI testing to help clinicians make informed diagnostic decisions. There are several considerations when evaluating guidelines and using them in clinical practice:

Test selection: Guidelines emphasize the importance of test selection based on the clinical context. NAATs have gained popularity due to their high sensitivity, but the guidelines stress the need for clinical correlation to avoid overdiagnosis.

Two-step testing: Many guidelines suggest a two-step approach, beginning with a highly sensitive test like NAAT

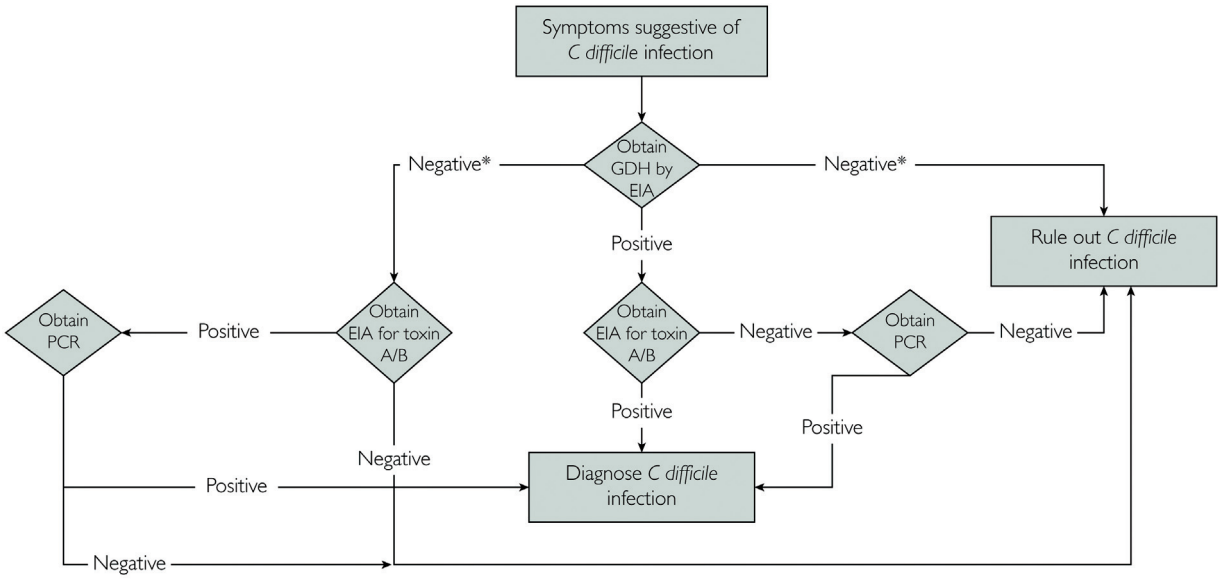

Fig. 1 Interpretation of a two-step testing algorithm for *C. difficile* (*Clostridioides* difficile) infection. EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; PCR, polymerase chain reaction. A negative GDH by itself is not used to rule out *C difficile* infection and is followed by an EIA for toxin A/B as the diagnostic kits check for both GDH and the *C difficile* toxins. Reprinted with permission.[6]

and confirming positive results with a toxin assay. This strategy balances sensitivity and specificity (►**Fig. 1**).[6]

Asymptomatic carriers: Guidelines acknowledge that asymptomatic carriers exist and recommend against screening for *C. difficile* in the absence of clinical symptoms.[7]

What Challenges and Controversies Exist in the Realm of *C. difficile* Testing, and How Do We Contextualize Evidence-Based Testing Strategies for CDI?

C. difficile testing faces several challenges and controversies that clinicians must navigate to improve diagnostic accuracy and patient care. Evidence-based testing strategies for CDI play a crucial role in improving patient outcomes, reducing healthcare costs, and promoting antimicrobial stewardship.

Overdiagnosis: NAATs have high sensitivity but do not differentiate between colonization and active infection. This leads to the overdiagnosis in asymptomatic carriers or patients with other causes of diarrhea. Clinicians must use judgment to avoid overtreatment.

Underdiagnosis: Toxin detection tests, such as EIAs, have lower sensitivity than NAATs and may miss cases of CDI, especially those caused by nontoxigenic strains or new toxin variants. This can result in underdiagnosis and delayed treatment.

Improved patient outcomes: Accurate CDI diagnosis ensures that patients with genuine infections receive appropriate treatment promptly. This reduces morbidity and the risk of severe complications, such as pseudomembranous colitis or toxic megacolon.

Asymptomatic carriers: Asymptomatic *C. difficile* carriers may be a source of transmission in healthcare. The significance of detecting *C. difficile* in the absence of symptoms is debatable. Testing asymptomatic individuals can lead to unnecessary treatment and potential harm.

Antimicrobial stewardship: Reducing inappropriate antibiotic use is a fundamental aspect of CDI prevention. Evidence-based testing strategies help identify situations where CDI is less likely and suggest avoiding testing. This contributes to the broader goal of antimicrobial stewardship by curbing antibiotic resistance and the risk of CDI.

Reduced healthcare costs: Evidence-based testing strategies help prevent the overdiagnosis of CDI, reducing the financial burden associated with unnecessary treatment and hospital isolation. Additionally, by guiding appropriate therapy, these strategies can lead to cost-effective patient management.

When Should and Should Not a Patient Be Retested for *C. difficile* Infection?

Testing for CDI should be performed in the context of clinical symptoms of the disease keeping the syndrome in mind. As is well known in the field, the risk of recurrent CDI after a primary infection can be 20 to 30% and this rate of recurrence can be more than 50% in patients with multiple infections. There is also a risk of postinfection irritable bowel syndrome that ranges between 20 and 30% after CDI has resolved.

1. Patients who do not exhibit symptoms of CDI after antibiotic treatment is completed should never be retested. The risk of false positive test can be up to 50%. Treatment of a false-positive test or a carrier state can lead to future CDI and does not confer any clinical benefit.
2. Patients who exhibit symptoms of postinfection irritable bowel syndrome after antibiotics for CDI are stopped should not be tested for CDI. The detailed symptom history to exclude postinfection irritable bowel syndrome is important period; these patients typically present with

diarrhea, alternating constipation or formed stools with or without abdominal cramps, with symptoms generally related to food intake.

3. Patients who have recurrence of CDI symptoms, that is, otherwise unexplained recurrent diarrhea would benefit from repeat testing for CDI.

Are There Any Emerging Technologies in *C. difficile* Testing That Show Promise for Improving Accuracy of Diagnosis?

The field of testing for CDI is evolving, with emergence of technologies showing promise for improving diagnostic accuracy. These emerging trends and technologies offer exciting opportunities to enhance CDI diagnosis. However, their clinical utility and cost-effectiveness need further evaluation before widespread adoption.

Ultrasensitive toxin detection: Tests are being developed to improve sensitivity of toxin bases assays.

Phenotypic testing: Phenotypic testing, which measures the metabolic activity of *C. difficile*, may help distinguish between colonization and active infection. It is an area of ongoing research.

Metagenomic sequencing: Metagenomic sequencing, which analyzes the entire gut microbiome, is gaining attention for its potential to detect *C. difficile* and other pathogens. This approach may offer higher sensitivity and specificity, as well as the ability to identify antibiotic resistance genes.

Potential Competing Interests

Research grants from Rebiotix / Ferring, Finch, Vedanta and consulting fees from Seres, Takeda, Immuron, Niche and ProbioTech Inc.

Ethical Statement

Not applicable.

Author Contribution

All authors contributed equally to the article.

Data Availability Statement

There are no data associated with this work.

Funding

SK receives research support from Rebioitx / Ferring, Vedanta, Finch, Seres and Pfizer. SK serves as a consultant for ProbioTech, Takeda and Rise.

Conflict of Interest

None declared.

Acknowledgments

None.

References

1 Lessa FC, Winston LG, McDonald LCEmerging Infections Program C. difficile Surveillance Team. Burden of Clostridium difficile infection in the United States. N Engl J Med 2015;372(24):2369–2370
2 Guh AY, Mu Y, Winston LG, et al; Emerging Infections Program Clostridioides difficile Infection Working Group. Trends in U.S. burden of *Clostridioides difficile* infection and outcomes. N Engl J Med 2020;382(14):1320–1330
3 Kelly CR, Fischer M, Allegretti JR, et al. ACG clinical guidelines: prevention, diagnosis, and treatment of Clostridioides difficile infections. Am J Gastroenterol 2021;116(06):1124–1147
4 Gupta A, Khanna S. Repeat Clostridium difficile testing. JAMA 2016;316(22):2422–2423
5 Guh AY, Hatfield KM, Winston LG, et al. Toxin enzyme immunoassays detect Clostridioides difficile infection with greater severity and higher recurrence rates. Clin Infect Dis 2019;69(10):1667–1674
6 Khanna S. My treatment approach to Clostridioides difficile infection. Mayo Clin Proc 2021;96(08):2192–2204
7 van Prehn J, Reigadas E, Vogelzang EH, et al; Guideline Committee of the European Study Group on Clostridioides difficile. European Society of Clinical Microbiology and Infectious Diseases: 2021 update on the treatment guidance document for Clostridioides difficile infection in adults. Clin Microbiol Infect 2021;27(Suppl 2):S1–S21

