Diagnostic Approaches for *Clostridioides difficile* Infection

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KEYWORDS

- *Clostridioides difficile* infection • Glutamate dehydrogenase
- Multistep testing algorithm • Pseudomembranous
- *Clostridioides difficile* infection diagnosis • Diagnostic stewardship

KEY POINTS

- Test only symptomatic patients. Diagnostic evaluation should be limited to patients with at least 3 unformed stools in 24 hours.
- Use multistep testing algorithms. Combining a sensitive test (eg, glutamate dehydrogenase or NAAT) with a specific test (toxin EIA) increases diagnostic accuracy by confirming toxin-mediated disease.
- NAAT alone is not sufficient. Polymerase chain reaction detects toxigenic strains but cannot distinguish colonization from active infection. Clinical correlation is essential.
- Avoid unnecessary repeat testing. Routine retesting, especially within 7 days of a negative result or after treatment (test of cure), should be avoided.

INTRODUCTION

Clostridioides difficile is an anaerobic, spore-forming, gram-positive bacillus that is a leading cause of health care-associated diarrhea and colitis. In the United States, *C difficile* infection (CDI) affects approximately 500,000 individuals annually and is associated with around 30,000 deaths.[1] Up to 35% of patients with an initial episode experience recurrence, with as many as 60% of those experiencing further episodes.[2] CDI represents a global challenge, with recurrence and misdiagnosis contributing to prolonged hospitalizations and escalating health care costs.[3,4] The average CDI-attributable cost per case is estimated at $21,448, increasing to $34,157 for hospital-onset cases.[5]

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Abbreviations	
ACG	American College of Gastroenterology
AI	artificial intelligence
CCNA	cytotoxicity neutralization assay
CDI	*Clostridioides difficile* infection
CI	confidence interval
EIA	enzyme immunoassay
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
GDH	glutamate dehydrogenase
IBD	inflammatory bowel disease
IDSA	Infectious Diseases Society of America
IFA	indirect fluorescent antibody
IL	interleukin
MALDI-TOF	matrix-assisted laser desorption/ionization-time of flight
NAAT	nucleic acid amplification test
PCR	polymerase chain reaction
PI-IBS	postinfection irritable bowel syndrome
SHEA	Society for Healthcare Epidemiology of America
SiMoA	single molecule array
TC	toxigenic culture
WGS	whole-genome sequencing

Early and accurate diagnosis through stool studies is essential to expedite treatment and prevent severe outcomes, including organ failure and death, which can occur in up to 14% of severe CDI cases.[6] Diagnostic costs vary by testing modality.

Despite diagnostic advancements, challenges persist, including distinguishing colonization from active infection, variability in test performance, and lack of standardization across institutions. One major challenge in diagnosing CDI is the absence of a single test that offers both high sensitivity and specificity. False negatives may lead to undertreatment and loss of clinician confidence in testing, prompting unnecessary empiric therapy that can worsen microbiome disruption and increase CDI risk. False positives may lead to unnecessary treatment, isolation, and diagnostic delay.

Major guidelines recommend multistep testing algorithms. Although combination testing strategies offer improved diagnostic accuracy over individual tests, they remain imperfect, as they are subject to interpretive ambiguities and interlaboratory variability.

Additionally, the variability in toxin gene sequences among *C difficile* strains and the presence of nontoxigenic strains complicate the diagnostic process. Whole-genome sequencing (WGS) has revealed that a nucleic acid amplification test (NAAT) alone has significant limitations in distinguishing true infection from colonization. It reveals phylogenomic equivalency between isolates from symptomatic and asymptomatic individuals, confirming that the same toxigenic strains circulate in both groups and that the presence of a toxigenic strain by NAAT does not equate to clinical disease.[7]

This article provides a comprehensive, clinically focused overview of CDI diagnosis, highlighting current testing algorithms, diagnostic pitfalls, and emerging strategies aimed at improving accuracy and reducing overdiagnosis.

WHEN TO TEST FOR *CLOSTRIDIOIDES DIFFICILE*: CLINICAL CRITERIA

All major guidelines, including American College of Gastroenterology (ACG), Infectious Diseases Society of America/Society for Healthcare Epidemiology of America (IDSA/SHEA), and European Society of Clinical Microbiology and Infectious Diseases (ESCMID), recommend testing for CDI only in symptomatic patients, typically defined as

those with 3 or more unformed stools in 24 hours and no alternative explanation, such as laxative use.[8–10] An important exception to the diarrhea threshold includes fulminant CDI, where patients may present with ileus or toxic megacolon. In such cases, rectal swabs may be used for testing. Clinical features prompting diagnostic consideration include watery diarrhea, abdominal pain, and systemic signs such as fever or leukocytosis.[9] It is important to assess the chronology of symptoms and contextual factors, such as recent antibiotic exposure or initiation of enteral feeding, to avoid testing patients with a more likely alternative explanation.[11]

Testing should generally be avoided in children under 1 year of age because of the high prevalence of asymptomatic colonization, which can lead to false-positive results.[12,13] In 1- to 2-year-old children, testing should not be performed unless other infectious and noninfectious causes are excluded. Current guidelines from ACG, IDSA/SHEA, and ESCMID discourage repeat testing within a short interval. Routine testing for cure in asymptomatic patients after treatment is not recommended, because patients can continue to shed *C difficile* even after symptom resolution.

DISTINGUISHING COLONIZATION FROM INFECTION: DIAGNOSTIC, CLINICAL, AND INFECTION CONTROL PERSPECTIVES

Distinguishing *C difficile* colonization from active infection is a clinically significant and diagnostically challenging task, with important implications for patient management and infection control. Colonized individuals may test positive on NAAT or toxigenic culture, but true infection is defined by new-onset diarrhea (at least 3 unformed stools in 24 hours) plus laboratory evidence of toxigenic *C difficile* (**Fig. 1**).

Colonized patients can shed spores and contaminate the environment, albeit at lower levels than symptomatic patients.[14] Some evidence supports the potential role of asymptomatic carriers in transmission.[15,16] However, major guidelines do not recommend treatment or routine isolation for colonized individuals.[9] As per these societies, contact precautions (eg, gloves, gowns, or dedicated equipment) are reserved for suspected or confirmed symptomatic CDI. Supporting this, a large randomized trial showed that universal gown and glove use in ICUs did not reduce CDI acquisition.[17]

That said, some institutions have piloted modified precautions (eg, gloves, soap-and-water hygiene, bed curtains, but no gowns or private rooms) with mixed results.[18–20] For symptomatic patients with discordant results (eg, NAAT-positive, toxin-negative), contact precautions are still advised, as ruling out CDI is difficult in such scenarios.[2] Routine carrier screening would significantly increase isolation-days, raising concerns about resource use and harms like falls or patient isolation.[21] Additionally, distinguishing transient from persistent colonization is complex. Serial NAATs cannot confirm chronic carriage.[22]

Transient colonization is defined as a single positive culture with negative cultures before and after, while persistent colonization requires multiple consecutive positive cultures. Clearance of colonization is typically defined as 2 consecutive negative cultures following a period of positivity. Persistent carriers often harbor higher organism burden and are at greater risk for developing CDI.[23,24] They also pose a higher transmission risk than transient carriers because of prolonged shedding.[2]

Serial perirectal or stool cultures are required to make distinction, as a single positive test cannot differentiate between transient and persistent colonization.[22] Molecular typing (eg, ribotyping or whole-genome sequencing) may confirm persistence of the same strain, but is not routinely performed outside of research or epidemiologic investigations.[25]

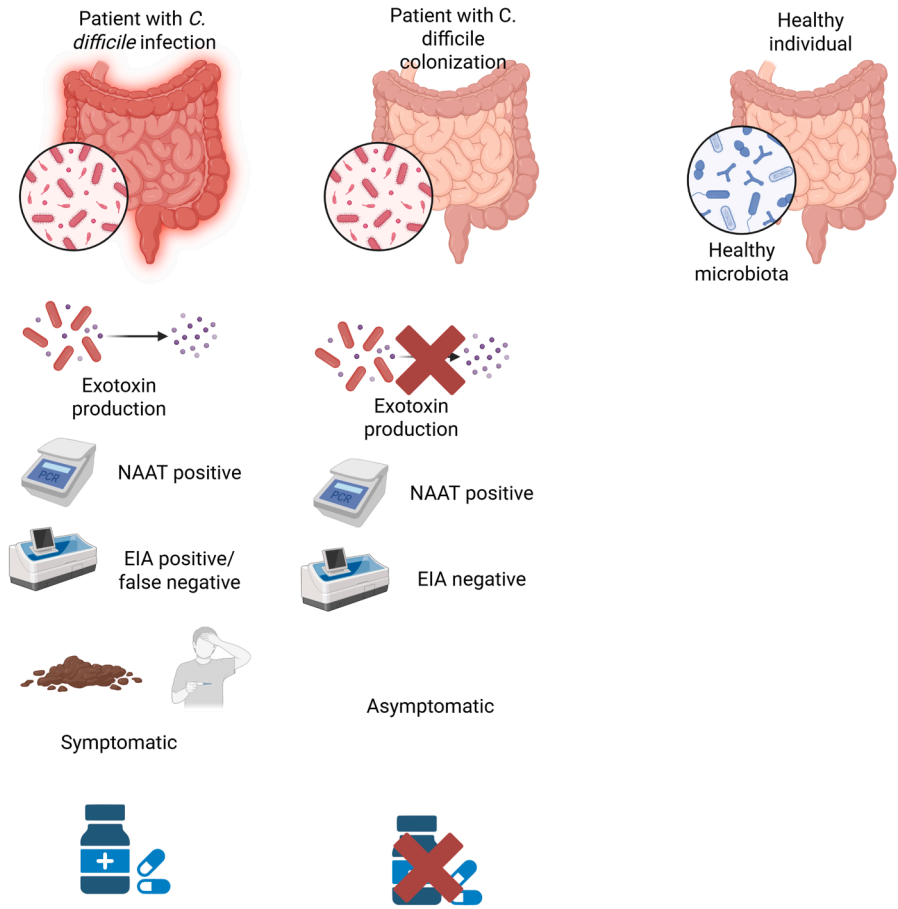

Fig. 1. Colonization versus *C difficile* infection. (*Created in* BioRender. Berry, P. (2025) https://BioRender.com/hpaabdz.)

Current guidelines from the ACG, IDSA/SHEA, ESCMID, and the American Society of Colon and Rectal Surgeons do not recommend routine screening or treatment of asymptomatic carriers, regardless of colonization duration, as eradication has not been shown to reduce CDI incidence and may disrupt the microbiome.[8–10,26,27]

From a diagnostic perspective, enzyme immunoassays (EIAs) for toxins A and B are useful in identifying active infection, as they detect free toxin in the stool, which is typically absent in colonized carriers. Toxin production is central to disease pathogenesis, with TcdA and TcdB glucosylating Rho-family GTPases, disrupting cytoskeletal structure and tight junctions, and inducing mucosal inflammation, epithelial damage, and pseudomembrane formation.[28,29]

In contrast, NAATs, although highly sensitive, detect only the genes encoding toxins and not active toxin production, making them unable to differentiate infection from colonization when used alone.[30] Accordingly, all major guidelines, ACG, IDSA/SHEA, and ESCMID, recommend multistep testing algorithms in symptomatic patients that pair a high-sensitivity screening test with a high-specificity confirmatory assay to optimize diagnostic accuracy and minimize overdiagnosis.[11] Ultimately, the presence of

clinical symptoms in conjunction with positive toxin or algorithmic testing remains the most reliable method for distinguishing infection from colonization.[31,32]

DIAGNOSTIC MODALITIES: INDIVIDUAL TEST PERFORMANCE

Over the past decade, *C difficile* diagnostic strategies have evolved from slow, labor-intensive methods to rapid molecular and now toward hybrid testing algorithms (**Table 1**).

Reference Tests

Historically, CDI diagnosis relied on reference methods such as cell cytotoxicity neutralization assay (CCNA) and toxigenic culture (TC).[38,39] The cytopathic effects of toxin B on cell lines, reversed by specific antitoxins, are assessed using CCNA.[40] In contrast, TC involves isolating organisms on selective media, followed by identification methods like MALDI-TOF (matrix-assisted laser desorption/ionization-time of flight) and confirmatory toxin or gene testing.[41,42] There is ongoing debate over which reference standard better reflects true disease.[43] CCNA reflects in vivo toxin activity and correlates more reliably with clinical outcomes, whereas TC detects a strain's potential to produce toxin in vitro, without distinguishing true infection from asymptomatic colonization. Studies show that patients testing positive by CCNA or toxin EIAs tend to have worse outcomes than those positive by TC alone, suggesting that TC may overdiagnose CDI by identifying colonized individuals. Despite its clinical relevance, CCNA is limited by poor standardization, sensitivity to sample handling, and technical demands. Both methods are labor-intensive and resource-consuming, making them impractical for routine clinical use.

These tests were gradually replaced by rapid assays, including EIA or single-molecule array assays for toxin A/B, EIA for glutamate dehydrogenase (GDH), and NAAT detecting toxin genes.

Toxin Enzyme Immunoassays

Toxin EIAs can help differentiate active infection from colonization because of their high specificity (approximately 99%) and rapid turnaround time (<2 hours). However, their variable sensitivity (57%–83%) limits reliability, especially in cases with low toxin levels, increasing the risk of false negatives.[9,44–46] As a result, they may fail to detect true infection in symptomatic patients and are affected by storage conditions. Unlike quantitative methods such as indirect fluorescent antibody (IFA), EIAs provide qualitative results. Newer ultrasensitive assays, like SiMoA (single molecule array), offer improved sensitivity but remain limited in availability.[47,48] They are often implemented in algorithms after an initial highly sensitive test.

Glutamate Dehydrogenase Assays

The GDH EIA is a rapid, cost-effective, and highly sensitive screening tool for CDI. It detects GDH, an enzyme produced by toxigenic and nontoxigenic *C difficile* strains and other microorganisms, which limits its specificity. Although its high negative predictive value makes it useful for ruling out CDI in symptomatic patients, it lacks the specificity to confirm infection and performs poorly as a stand-alone test, particularly in low-prevalence settings where its positive predictive value is low.[9,12,44,49]

Nucleic Acid Amplification Tests

Since 2010, NAATs have seen widespread adoption, with nearly half of US laboratories incorporating them because of their high sensitivity.[50] These assays—including

Table 1
Diagnostic modalities for *Clostridioides difficile* infection

Test Modality	Sensitivity (%)	Specificity (%)	Typical Use and Comments	Limitations
Toxigenic Stool Culture	Approaching 100%	Approaching 100%	The most sensitive and specific method for detecting *C difficile* Can differentiate between toxigenic and nontoxigenic strains	Labor-intensive and time-consuming (48–72 h for results) Although highly accurate, toxigenic culture does not differentiate between colonization and active infection[1]
CCNA	85%-90%[2]	100%	Highly specific, making it a reliable confirmatory test Considered a reference method for detecting C difficile toxins, providing a benchmark for evaluating other diagnostic tests Directly detects the cytotoxic effect of toxins, which is crucial for confirming active infection rather than mere colonization	Labor-intensive and requires significant technical expertise Time-consuming typically requiring 24 to 48 h Relies on the subjective interpretation of cytopathic effects
Stool toxin EIA for Tcd A and B	48% (95% CI [confidence interval]: 0.41–0.55)[3,4]	95% (95% CI: 0.94–0.96)	Highly specific for CDI, making positive results strongly indicative of active infection Provide results in <2 h[5] Cost-effective making them accessible in resource-limited settings[6]	Lower sensitivity, which can lead to underdiagnosis. Sensitivity of EIAs can be impacted by specimen handling and the quality of the assay used.

GDH antigen (stool)	91% (95% CI: 0.871–0.940)[8]	91% (95% CI: 0.892–0.928)	A reliable initial screening tool for CDI High negative predictive value (a negative GDH result can reliably exclude CDI) Rapid and cost-effective[9]	Poor specificity for toxigenic strains Inability to differentiate between colonization and infection may yield false positives Require confirmation with either toxin EIA or NAAT
NAATs	93%-100%	Approximately 95%	Highly sensitive, making them effective for detecting the presence of the *C difficile* toxin B gene, which helps in identifying toxigenic strains. Provides rapid results High negative predictive value.	Cannot distinguish between colonization and active infection, leading to potential overdiagnosis and overtreatment, especially in asymptomatic carriers Detects both viable and nonviable organisms, as well as nontoxin-producing strains, which can complicate the interpretation of positive results More expensive and requires specialized equipment and expertise, which may limit their availability in resource-constrained settings

From.[33–37]

polymerase chain reaction (PCR), helicase-dependent, and loop-mediated isothermal amplification—primarily target conserved regions of the *tcdA* and *tcdB* toxin genes, and some also detect binary toxin (*cdt*), and *tcdC* mutations associated with hypervirulent strains like ribotype 027/NAP1 detect only the presence of toxin genes, not active toxin production, making them unable to distinguish true infection from asymptomatic colonization. This limitation raises concerns about overdiagnosis, particularly when NAATs are used without clinical correlation or diagnostic stewardship.[9,51–54] Although PCR cycle thresholds may correlate with toxin burden and disease severity, their predictive value remains limited and varies by platform.[55] The initial surge in CDI diagnoses following widespread NAAT adoption underscored the need to reintroduce toxin detection into diagnostic algorithms.[56]

GUIDELINE RECOMMENDATIONS

Although rapid assays for *C difficile* are convenient, their use as standalone tests can lead to overdiagnosis, as GDH EIA and NAAT do not directly correlate with symptoms. To improve diagnostic accuracy, guidelines from ESCMID, IDSA, and ACG recommend multistep algorithms that begin with a sensitive screen (GDH EIA or NAAT) followed by a confirmatory toxin assay, leveraging high negative and positive predictive values when used sequentially.[8–10,44] Toxigenic culture, although inefficient for routine screening, retains utility in resolving ambiguous results.

There is no major discordance among these societies regarding the properties or recommended use of these assays, although some allow NAAT-only testing in specific, controlled clinical contexts. The IDSA/SHEA permits NAAT-only testing in specific settings—restricted to unformed stool from symptomatic patients, with strict clinical criteria in place. The American Society of Colon and Rectal Surgeons also allows NAAT-only testing, but only when multistep or toxin-based testing is unavailable, and results must be interpreted cautiously. In contrast, ESCMID and ACG discourage NAAT-only strategies because of the risk of overdiagnosing colonization.

DIAGNOSTIC ALGORITHMS IN CLINICAL PRACTICE

Multistep diagnostic algorithms have become the primary approach for diagnosing CDI in US hospitals, with 59% of laboratories adopting these protocols. The most widely used strategy involves an initial combination GDH and toxin EIA, followed by NAAT when results are discordant. NAAT alone is employed by 26% of laboratories, while 22.5% use NAAT followed by toxin EIA. Standalone use of toxin EIA is uncommon, reported by only 5.4% of laboratories.[57]

The Glutamate Dehydrogenase Plus Toxin Enzyme Immunoassay Algorithm

One widely used 2-step approach involves initial screening with GDH, a sensitive marker for *C difficile* presence, followed by toxin EIA to confirm active toxin production (**Fig. 2**). If GDH is negative, CDI is unlikely, and no further testing is required. If GDH is positive, toxin EIA is performed. If both GDH and toxin are positive, that is a conclusive positive, as an organism is present, and toxin is detected. If both are negative, CDI is ruled out.

The most diagnostically challenging scenario is GDH positive and toxin negative, which may indicate either colonization, as GDH is highly sensitive but not specific, or true CDI with undetectable toxin levels, as toxin EIA is highly specific but not sensitive. In such cases, a reflex NAAT is often used. A positive NAAT in this context suggests CDI, whereas a negative result makes infection unlikely and points toward a false-positive GDH test. Clinical correlation is critical in the case of discordant results.

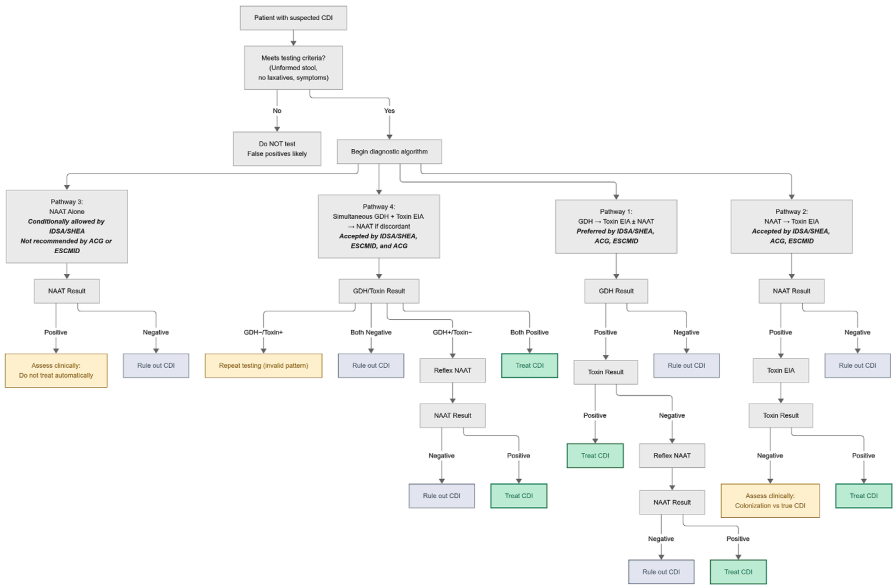

Fig. 2. Diagnostic algorithms for *C difficile* infection as recommended by major guidelines. (*Created in* BioRender. Berry, P. (2025) https://BioRender.com/ek2mvlw.)

If the patient's symptoms are classic for CDI and no alternate cause is found, most clinicians treat presumptively. However, if the clinical picture is ambiguous, some providers may opt for repeat testing or close monitoring rather than immediate treatment.

The Simultaneous 2-step Algorithm (Screening with Glutamate Dehyrogenase and Toxin Enzyme Immunoassay)

This approach tests for both GDH and toxin A/B at the initial step, using a combined assay. If both are negative, CDI is unlikely; if both are positive, CDI is confirmed. Discordant results require further evaluation. A positive GDH and negative toxin EIA may indicate colonization or low-level toxin production. A negative GDH with a positive toxin result is considered invalid and warrants repeat testing.

Nucleic Acid Amplification Test Followed by Toxin Enzyme Immunoassay

This alternative 2-step strategy starts with NAAT because of its high sensitivity and negative predictive value. If NAAT is negative, CDI is excluded. If NAAT is positive, toxin EIA is used as a confirmatory step. An NAAT-positive/EIA positive result confirms active infection. However, if toxin is negative (NAAT+/EIA−), the result enters a diagnostic gray zone. This pattern may indicate colonization or low-level toxin production and should be interpreted in clinical context. NAAT positive/EIA negative patients often have outcomes similar to patients without CDI if left untreated, implying many such cases may be carriers or have another diarrhea etiology, with *C difficile* being just a bystander.[58] A meta-analysis showed that while NAAT-positive/EIA-positive and NAAT-positive/EIA-negative patients had similar mortality and complication rates, toxin-positive patients had a higher likelihood of severe disease and recurrence.[59,60]

Nucleic Acid Amplification Test Alone

The use of NAAT alone may be acceptable under the IDSA/SHEA guidelines, but only if institutional protocols ensure appropriate patient selection, such as excluding laxative

use and confirming clinically significant diarrhea. If such safeguards are not in place, IDSA/SHEA recommends a multistep algorithm. In contrast, both the ACG and ESCMID discourage NAAT-alone strategies because of the risk of overdiagnosis from asymptomatic colonization.[8–10,44]

EVOLVING STRATEGIES AND KEY CONSIDERATIONS

In summary, diagnostic strategies have evolved from reliance on EIAs to widespread adoption of PCR-based testing, and now toward balanced multistep algorithms. The overarching principle is that no single test is perfect for CDI. Multistep algorithms are recommended over using a single NAAT alone in settings without preagreed stool submission criteria, and over using a toxin test alone when such criteria are in place. Testing for CDI in specific populations, such as patients with inflammatory bowel disease (IBD) experiencing a flare with diarrhea, is recommended, and a 2-step algorithm is preferred in this group because of the common occurrence of colonization.

Timing also plays a crucial role in test reliability. Empiric treatment before specimen submission may suppress toxin levels, leading to false-negative results. Currently, no universally accepted diagnostic algorithm exists, resulting in significant interinstitutional variability. Differences in test selection, interpretation, particularly in NAAT-positive/EIA-negative cases, and diagnostic stewardship practices complicate clinical decision making.

This diagnostic heterogeneity contributes to inconsistencies in CDI reporting and hinders the comparability of incidence data across health care systems.[61] Establishing consensus-driven, clinically relevant protocols is essential for improving accuracy, guiding treatment, and standardizing CDI diagnosis.

EMERGING BIOMARKERS AND NOVEL DIAGNOSTICS IN *CLOSTRIDIOIDES DIFFICILE* INFECTION: ENHANCING CLINICAL DECISION MAKING

A growing array of ancillary and emerging diagnostic methods is being explored.[62] Ultrasensitive immunoassays capable of detecting low-level toxin production may help resolve discordant cases, particularly when NAAT is positive but toxin EIA is negative, by improving specificity and reducing overdiagnosis.

Novel approaches such as CRISPR-Cas12a systems, real-time impedance cytometry, and lateral flow multiplex platforms show promise in distinguishing active infection from asymptomatic carriage.

In parallel, inflammatory biomarkers such as fecal calprotectin and lactoferrin have been associated with intestinal inflammation in CDI but lack the specificity required for routine clinical use.[63] More promising are cytokines like interleukin (IL)-1β, IL-6, IL-8, and G-CSF, which have been linked to severe disease and adverse outcomes. Elevated stool or serum levels of IL-1β and G-CSF, in particular, may help identify patients at higher risk of complications, although they are not yet standard in clinical practice.[64]

Additionally, microbiome profiling revealing dysbiosis patterns such as *Enterococcus* enrichment and *Ruminococcus* depletion may offer future tools for predicting CDI risk, especially before antibiotic exposure.[65] Although these innovations are not yet ready for routine clinical implementation, they represent important steps toward more precise, individualized CDI management.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis for patients presenting with acute diarrhea and abdominal cramping, particularly following antibiotic exposure or hospitalization, must include

CDI. However, a range of other etiologies can mimic or coexist with CDI and should be considered. Enteric infections such as *Salmonella*, *Shigella*, *Campylobacter*, *Escherichia coli*, and norovirus can present similarly, and multiplex stool PCR may aid in identifying alternative pathogens.[66]

IBD flares closely resemble CDI and often warrant testing, especially as IBD increases susceptibility to CDI.[67] Many medications (eg, laxatives, stool softeners, metformin, certain chemotherapies, or magnesium-containing antacids) can cause diarrhea. A thorough medication review is needed. If a patient is on a laxative regimen, holding it to see if diarrhea improves is wise before testing for CDI.[68]

DISTINGUISHING *CLOSTRIDIOIDES DIFFICILE* INFECTION RECURRENCE FROM POSTINFECTIOUS SYNDROMES

It is also essential to differentiate CDI recurrence from postinfection irritable bowel syndrome (PI-IBS). By definition, recurrence is at least 3 loose stools with a positive *C difficile* test (typically toxin-positive) occurring within 2 to 8 weeks of initial infection. Chronic, intermittent diarrhea and cramping could be PI-IBS rather than an ongoing infection. Particularly after a treated CDI, up to 25% of patients develop PI-IBS, which can manifest as alternating diarrhea and constipation and can be mistaken for relapsing infection.[69] Clues to a noninfectious etiology include an atypical course (eg, long history of diarrhea predating antibiotic use, or symptoms that improve little with appropriate CDI therapy). When patients fail to improve after 4 to 6 days of appropriate therapy, clinicians should reassess for refractory CDI or alternative diagnoses.

CLINICAL OUTCOMES/LONG-TERM RECOMMENDATIONS

Several studies have examined the clinical significance of different CDI diagnostic profiles, particularly comparing NAAT-positive/EIA-positive with NAAT-positive/EIA-negative cases. Consistently, toxin-positive patients (NAAT+/EIA+) show higher recurrence rates (approximately 20%) and more severe disease than toxin-negative cases (approximately 11%).[70]

Thirty-day mortality rates were overall similar between groups; however, a notable difference emerged within the NAAT-positive/EIA-negative subset: patients who received treatment had a lower mortality rate (5.0%) than those who remained untreated (14.9%).[70] This suggests that while many NAAT-positive/EIA-negative cases may represent colonization rather than true infection, a subset may still benefit from targeted therapy. This discrepancy may in part reflect the low sensitivity of EIAs, which may miss clinically significant cases with low-level toxin production.

Severe CDI-related complications are rarely observed in NAAT-positive/EIA-negative patients, who, even when untreated, often experience clinical courses indistinguishable from those without CDI.[9] In contrast, toxin-positive individuals, regardless of NAAT status, are more likely to have true, clinically significant CDI.

Supporting this, Polage and colleagues found that only 45% of PCR-positive patients had detectable toxins. Toxin-negative individuals had lower bacterial loads, less severe symptoms, shorter diarrhea duration, and reduced fecal inflammation. No CDI-related complications were observed in the NAAT-positive/EIA-negative group, compared with a 7.6% complication rate in toxin-positive patients, and mortality was markedly lower (0.6% vs 8.4%, P=.001).[51] These findings reinforce concerns over overdiagnosis when NAAT is used as a stand-alone diagnostic tool.

DIAGNOSTIC STEWARDSHIP: REDUCING OVERDIAGNOSIS

To reduce false positives and minimize unnecessary CDI therapy, many institutions have adopted diagnostic stewardship strategies. These efforts focus on restricting testing to patients with true clinical suspicion and limiting repeat testing within short timeframes. Electronic health record tools, including clinical decision support alerts and order-entry hard stops have proven effective. For instance, 1 hospital implemented computerized decision support that required documentation of at least 3 unformed stools and automatically blocked test orders for patients receiving laxatives. This intervention led to a substantial reduction in inappropriate *C difficile* testing, from 31.1% to 11.0%, representing a 20.1% absolute decrease.[71]

Clinician education and feedback strategies can also reduce hospital-onset CDI.[72]

The overarching goal is to promote the right test for the right patient, avoiding unnecessary testing in low pretest probability scenarios and thereby minimizing false-positive diagnoses. Such misdiagnosis can result in unwarranted antibiotic use, extended hospitalizations, and increased health care costs.

SUMMARY/DISCUSSION

Accurate diagnosis of CDI remains a cornerstone of effective clinical management and infection control. The diagnostic process must integrate clinical suspicion with confirmatory testing for toxigenic *C difficile*, while recognizing the limitations of current assays. NAATs offer high sensitivity but risk overdiagnosis because of detection of colonization, whereas toxin EIAs provide greater specificity but may miss active cases because of low sensitivity. To overcome these limitations, multistep testing algorithms, combining GDH and toxin EIA with arbitration by NAAT, are now widely endorsed as the preferred diagnostic strategy.

To optimize diagnostic accuracy, clinicians should adhere to key principles of testing only unformed stools from symptomatic patients, avoiding testing in patients on laxatives, and interpreting discordant results in a clinical context. Diagnostic stewardship is crucial, particularly in institutions using NAAT alone, where strict pretest criteria are necessary to minimize misclassification.

Improved diagnostics have far-reaching implications for patient care. Accurate and timely diagnosis guides targeted treatment, reduces unnecessary antibiotic exposure, and prevents CDI transmission. Moreover, minimizing diagnostic errors supports antimicrobial stewardship goals, curbs overtreatment, and preserves gut microbiota. Future advancements, including host-response biomarkers, multiomics, and artificial intelligence (AI)-driven algorithms, may enhance precision and personalization in CDI diagnostics.

CLINICS CARE POINTS

- Test only symptomatic patients. CDI testing should be limited to individuals with at least 3 unformed stools in 24 hours, in the absence of alternate explanations. Avoid testing formed stools unless ileus is suspected.
- Avoid empiric antibiotic treatment before stool collection. Empiric therapy may suppress toxin levels and lead to false-negative results. Always collect stool before initiating treatment whenever feasible.
- Use multistep algorithms for accuracy. Combine GDH antigen, toxin EIA, and NAAT testing to balance sensitivity and specificity, avoiding overdiagnosis from NAAT-only approaches.

- Avoid repeat testing within 7 days. Retesting rarely changes management and can lead to misinterpretation of disease course, particularly in previously treated patients.
- Distinguish colonization from infection. Positive NAAT with negative toxin should prompt clinical correlation rather than automatic treatment initiation, especially in immunocompromised or hospitalized patients.
- Watch for diagnostic pitfalls. EIAs may miss low-level toxin producers, underestimating disease in truly infected patients.
- Standardize diagnostic approaches. Institutional algorithms and provider education are key to reducing variability and improving diagnostic stewardship.

DISCLOSURES

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