DETECTION OF TOXIGENIC CLOSTRIDIUM DIFFICILE

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INSTRUCTIONS FOR USE
Physician Decision Support (PDS) is a lab ordering tool operated by BeaconLBS. This Clinical Guideline supports the Questions and Answers that appear in PDS for tests referenced in this document. UnitedHealthcare reserves the right, in its sole discretion, to modify its Clinical Guidelines as necessary. This Clinical Guideline is provided for informational purposes. It does not constitute a Medical Policy or medical advice.

GUIDELINES

A single stool specimen testing for toxigenic Clostridium difficile infection (CDI). It is recommended that NAT testing be the screening test of choice.

Indications for testing for toxigenic CDI in stool include:

- Clinical symptoms of CDI, including 3 or more diarrheal stools (watery, loose, or unformed) in a 24-hour period; AND at least one of the following conditions or risk factors:
  - Received antibiotics or drugs with antimicrobial activity (such as anti-cancer agents or antiretrovirals) within the previous 8-12 weeks;
  - Clinical presentation of CDI infection, or other symptoms of severe and/or complicated CDI including: abdominal tenderness, ileus or significant abdominal distention, hypotension, bloody stools, fever (≥38.5°C.), tenesmus, organ failure, mental status changes, severe or prolonged symptoms gastrointestinal symptoms, abnormal laboratory markers (including: leukocytosis, high creatinine, hypoalbuminemia, high lactate);
  - Co-morbid conditions (immune compromised; inflammatory bowel disease, pregnancy or peripartum women, recent hospitalization).
Additionally, for the laboratory testing of CDI:

- Repeat testing is not recommended.
- Testing for cure is not recommended.
- Nucleic acid amplification tests (NAAT) for *C. difficile* toxin genes such as PCR are superior to toxins A + B EIA testing as a standard diagnostic test for CDI.
- Glutamate dehydrogenase (GDH) screening tests for *C. difficile* can be used in two- or three-step screening algorithms with subsequent toxin A and B EIA testing, but the sensitivity of such strategies is lower than NAATs.

These recommendations are based on guidelines from the American Society of Gastroenterology (published in the American Journal of Gastroenterology) and the American Society for Microbiology.

**BACKGROUND**

Infectious diarrheal diseases (IDD) are caused by bacteria, parasites, or viruses and remain a major global health problem. It is the second leading cause of morbidity and mortality worldwide. The fecal microbiome is an incredibly complex milieu normally made up of anaerobes and facultative bacteria. Clinical diagnosis of the etiology of diarrhea cannot be made with certainty because the manifestations of illnesses accompanying diarrhea overlap. Many pathogenic bacteria, as well as viruses and parasites, have similar clinical presentations, therefore correct diagnosis before treatment must be made. Antibiotic therapy is indicated in many severe cases for diarrhea caused by bacterial pathogens in order to shorten the course of the illness and organism excretion, avoid morbidity and mortality, and prevent complications.

*C. difficile* is a widely disseminated anaerobic, Gram-positive spore-forming bacillus that produces 2 exotoxins, toxin A and toxin B, which cause damage to the large intestine, resulting in diarrhea among infected patients. It is an opportunistic pathogen, affecting primarily the elderly and the immunocompromised. It inhabits the human intestinal tract in 3% of healthy adults and in about 20-40% of hospitalized patients. Up to 50% of neonates may be colonized with toxigenic *C. difficile*. CDI rates doubled from 2000 – 2003 and it has become the most common cause of IDD in hospitals, accounting for 15–39% of antibiotic-associated diarrhea cases. Nosocomial infection beginning 3-4 days after hospitalization and antibiotic therapy is commonly due to *C. difficile*, and accounts for almost all cases of pseudomembranous colitis.

Recently, the trend has moved to increasing numbers of younger, post-transplant, immunocompromised patients. In addition, increasing numbers of patients with inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn’s Disease (CD) have *C. difficile* infections characterized by longer hospitalizations with more surgical procedures and higher mortality.

**Pathogenic process**

The pathogenic process of *C. difficile* infection starts with initial colonization followed by the production of two distinct exotoxins, Toxin A and B (TcdA and TcdB), as well as an additional toxin called binary toxin (CDT) which is found in some hypervirulent strains of *C. difficile*. Toxin A is an enterotoxin that can cause extensive mucosal damage and is an activator of mast cells and neutrophils, which trigger inflammatory mediators. Toxin B is a
cytotoxin that is known to cause morphological changes in tissue culture cells. Both are potent cytotoxins that disrupt cytoskeletal integrity. In addition, toxins A and B initiate an extensive inflammatory cascade that causes increased damage to host tissues resulting in fluid exudation.

**Clinical features**

An episode of CDI is characterized by diarrhea with an increase in stool frequency (watery, loose, or unformed stools that take the form of the container) stools more than three times/day), fever, leukocytosis, nausea, abdominal pain, and tenderness with non-bloody stools. The severity of CDI may range from mild (uncomplicated diarrhea) to moderate (fever, profuse diarrhea, abdominal pain, and leukocytosis) and, in extreme instances, severe (pseudomembranous colitis, toxic megacolon, and sepsis) disease. An important exception to the unformed stools is the very rare case where a patient has ileus (obstruction of the intestine due to paralysis of the intestinal muscles) without diarrhea.

Unusual disease manifestations associated with CDI include extra-intestinal infections, ileal infections, post-colectomy enteritis, reactive arthritis, and bacteremia. Risk factors include: age above 65 years, immunosuppression, cancer, gastrointestinal disorders, previous antibiotic use, and previous hospitalization, the use of proton pump inhibitors, inflammatory bowel disease, and residence in extended-care facilities. Recurrence (single and multiple) may occur in 15-35% (and higher in some studies) of infections and may be due to new more virulent strains (including the NAP1/027 strain).

### CLINICAL EVIDENCE

Early, rapid, and accurate diagnosis is crucial to treat CDI to avoid morbidity and mortality. CDI is a clinical diagnosis confirmed by detection of toxigenic *C. difficile* in stool. Due to high levels of asymptomatic carriage for toxigenic *C. difficile* among patients in hospitals and long term care facilities (up to 50% carriage rate), it is essential that only specimens from CDI symptomatic patients are submitted for laboratory testing. It is suggested that: only submit diarrheal stools; use one sample only, as several do not increase yield; and test for diagnosis only and not test of a cure.

Presently there is no single simple, inexpensive, rapid, sensitive, and specific test for diagnosing *C. difficile*. Although uncertainty about the optimal approach to diagnose *C. difficile*–associated disease exists, there are multiple tests available to detect toxigenic *C. difficile* in stool:

- The tissue culture cytotoxin neutralization assay, in which the cytopathic effect (CPE) (rounding of the cells) of toxin B stool filtrate is observed, is considered the “gold” standard (but is less sensitive than culture) for establishing the diagnosis of *C. difficile* infection. Specificity is confirmed when the cytotoxic action of the toxin is abolished after the stool filtrate is mixed with antitoxin. Although this test can be very sensitive (67-100%) and specific (>97%), the turnaround time can be slow (up to 48-72 h), and results can be variable between laboratories.
- Enzyme immunoassay (EIA) detects the presence of toxins A and B in stool. These assays are inexpensive, accurate, easily performed, and rapid but have low sensitivity (60-80%) as compared to the cytotoxin neutralization assay, nucleic acid amplification tests (NAATs), and toxigenic culture. Although multiple samples increase yield, it is not recommended. A negative result does not rule out the presence of toxin.
• Detection of the *C. difficile* common antigen glutamate dehydrogenase (GDH) as an initial screening in a two-step algorithm has become more popular, despite variable sensitivity, but specimens that test positive must be confirmed by a toxin detection method, either toxin EIA, cytotoxin neutralization, or NAAT.

• Toxigenic anaerobic stool culture is the most sensitive test but requires pre-treatment of the stool specimen with heat or alcohol, detection and identification of the *C. difficile* bacteria on pre-reduced anaerobic culture media, culture of the isolated *C. difficile* in broth medium, and finally detection of *C. difficile* toxins A and/or B by either toxin A/B EIA or tissue culture cytotoxin neutralization assay.

• Commercially available NAATs detect toxigenic *C. difficile* strains, where a portion of the toxin A or B gene is targeted and amplified. Real time polymerase chain reaction (RT-PCR) is rapid, sensitive (86- 100%), and specific (93-98%); LAMP (Loop Mediated Isothermal Amplification) technology (98% sensitivity and specificity) is now also available for use.\(^5,7,14\)

Even with newer test methodologies, false negatives and false positives can still occur.\(^9\) Additionally, there is no test to determine the response to therapy. A summary of the tests available is described in Table 1.

**Table 1. Tests to detect toxigenic *Clostridium difficile* in stool\(^6\)**

<table>
<thead>
<tr>
<th>Test</th>
<th>Substance detected</th>
<th>Time required</th>
<th>Sensitivity %*</th>
<th>Specificity %*</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Culture Cytotoxin Neutralization Assay</td>
<td>Toxin B</td>
<td>1-3 days</td>
<td>67-100</td>
<td>97-100</td>
<td>Not standardized; labor intensive; slow turnaround time</td>
</tr>
<tr>
<td>Toxigenic Anaerobic Stool Culture</td>
<td><em>C. difficile</em> bacteria and toxins A and/or B</td>
<td>3-7 days</td>
<td>&gt;90</td>
<td>95-97</td>
<td>Not standardized; labor intensive; slow turnaround time; must also do toxin test</td>
</tr>
<tr>
<td>EIA A/B Toxin</td>
<td>Toxins A+B</td>
<td>Hours</td>
<td>60-80</td>
<td>91-99</td>
<td>False negatives; poor positive predictive value; common in US; rapid; easy</td>
</tr>
<tr>
<td>EIA GDH</td>
<td><em>C. difficile</em> common antigen</td>
<td>Hours</td>
<td>85-100</td>
<td>76-90</td>
<td>Variable sensitivity; high negative predictive value; will detect non-toxigenic strains; must also do toxin test</td>
</tr>
<tr>
<td>NAATs</td>
<td><em>C. difficile</em> toxin gene</td>
<td>Hours</td>
<td>77-100</td>
<td>93-100</td>
<td>Expensive; high sensitivity and specificity</td>
</tr>
</tbody>
</table>

EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; NAATs, nucleic acid amplification tests

*Reported data for sensitivity and specificity are based on multiple reports. It is assumed that 20%-30% of hospitalized patients are colonized with *C. difficile* and that 80% of strains are toxigenic. Clinical correlations are always important but are especially important with NAAT and EIA tests for GDH.

**GUIDELINES AND RECOMMENDATIONS**

*American College of Gastroenterology*\(^7\)
The American College of Gastroenterology recently updated guidelines in 2013 that cover a range of recommendations from diagnostic testing and management of patients with CDI. The recommendations for diagnostic testing include:

- Only stools from patients with diarrhea should be tested for C. difficile
- Nucleic acid amplification tests (NAAT) for C. difficile toxin genes such as PCR are superior to toxins A +B EIA testing as a standard diagnostic test for CDI.
- Glutamate dehydrogenase (GDH) screening tests for C. difficile can be used in two- or three-step screening algorithms with subsequent toxin A and B EIA testing, but the sensitivity of such strategies is lower than NAATs.
- Repeat testing should be discouraged.
- Testing for cure should not be done.

The new guidelines clinically defined CDI disease as:

- Mild-to-moderate disease
  - Diarrhea plus any additional signs or symptoms not meeting severe or complicated criteria

- Severe disease
  - Serum albumin < 3 g / dl plus ONE of the following:
    - WBC ≥ 15,000 cells / mm 3 or
    - Abdominal tenderness

- Severe and complicated disease
  - Any of the following attributable to CDI:
    - Admission to intensive care unit for CDI
    - Hypotension with or without required use of vasopressors
    - Fever ≥ 38.5 °C
    - Ileus or significant abdominal distention
    - Mental status changes
    - WBC ≥ 35,000 cells / mm 3 or < 2,000 cells / mm 3
    - Serum lactate levels >2.2 mmol / l
    - End organ failure (mechanical ventilation, renal failure, etc.)

These updated guidelines also recognized several patient groups that are either at an elevated risk for acquiring the infection or suffering adverse outcomes from CDI:

- Patients who received possible inciting antimicrobial agents;
- Patients with a recent hospitalization;
- Patients with inflammatory bowel disease (IBD), including those with an ileostomy or an ileo-anal pouch following colectomy including:
  - All patients with IBD hospitalized with a disease fl are should undergo testing for CDI.
  - Ambulatory patients with IBD who develop diarrhea in the setting of previously quiescent disease, or in the presence of risk factors such as recent hospitalization, or antibiotic use, should be tested for CDI.
  - In patients who have IBD with severe colitis, simultaneous initiation of empiric therapy directed against CDI and treatment of an IBD fl are may be required while awaiting results of C.
difficile testing.

- In patients with IBD, ongoing immunosuppression medications can be maintained in patients with CDI. Escalation of immunosuppression medications should be avoided in the setting of untreated CDI.
- Patients with IBD who have a surgically created pouch after colectomy may develop CDI and should be tested if they have symptoms.
  - Patients with underlying immunosuppression (including malignancy, chemotherapy, corticosteroid therapy, organ transplantation, and cirrhosis/chronic liver disease) as this condition increases the risk of CDI, and such patients should be tested if they have a diarrheal illness;
  - Pregnant women and women in the peripartum period with diarrheal illness.

*American Society for Microbiology (ASM) Guidelines*¹⁴

The ASM offers a written policy document for the laboratory detection of toxigenic *C. difficile* that describes various guidelines including which patients to test for toxigenic *C. difficile*, which tests are recommended and in which order, and when repeat testing is appropriate.¹⁴ ASM has strict guidance on limiting the testing for *C. difficile* to those patients who have >3 non-formed stool specimens in 24 hours. In addition, ASM no longer recommends the toxin A/B EIA for standalone diagnosis and laboratories should instead use glutamate dehydrogenase antigen assays (with positive tests confirmed) and/or NAAT testing for diagnostic screening. The only repeat testing that ASM deems warranted is during clinical relapse.

**US FOOD AND DRUG ADMINISTRATION (US FDA)**

There are several FDA approved EIA and PCR based assays for the detection of *C. difficile*. If a test is used that has not been cleared or approved by the FDA, the performing institution must determine the performance characteristics.

**CENTERS FOR MEDICARE AND MEDICAID SERVICES (CMS)**

For Medicare populations, CMS does not pay for screening procedures (tests) performed in the absence of signs or symptoms. (Section 1862(a)(7) of the Social Security Act)

There are several CMS policies that apply to *C. difficile* toxin detection. In some cases, CMS reimbursement is limited to a certain number of tests for a patient per year. Physicians should consult their state’s regulations.

**APPLICABLE CODING**

<table>
<thead>
<tr>
<th>CPT Code</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>87324</td>
<td>Infectious agent antigen detection by enzyme immunoassay technique, qualitative or semiquantitative, multiple-step method; <em>Clostridium difficile</em> toxin(s)</td>
</tr>
<tr>
<td>87493</td>
<td><em>C. difficile</em> amplified probe</td>
</tr>
</tbody>
</table>
REFERENCES


## POLICY HISTORY/REVISION HISTORY

<table>
<thead>
<tr>
<th>Date</th>
<th>Action/Description</th>
</tr>
</thead>
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