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Abstract

*Clostridium difficile* infection (CDI) is a growing nosocomial and public health problem. Accurate diagnosis of CDI is essential for optimal treatment and prevention but continues to be challenging. Clinically, the disease can be diagnosed by symptoms of diarrhea accompanied by abdominal cramps, leukocytosis, fever, nausea, anorexia, malaise, dehydration and delirium. CDI may be diagnosed by colonoscopy to look for multiple yellow-white friable plaques or by computed tomographic (CT) scan when diarrhea is absent. Abdominal plain films may also demonstrate small bowel dilatation. *C. difficile* grows on selective media providing a low cost method but it requires a follow-up toxin testing. Toxigenic culture is considered a gold standard for CDI diagnosis as it combines both culture and toxin detection. *C. difficile* toxins can be detected in the fecal samples by tissue culture with cell lines such as McCoy, MRC-5 and Vero and as little as 1.0 pg of toxin B can be detected. Enzyme immunoassay (EIA) to detect toxin A or both toxins A and B in stool is quite popular. Glutamate dehydrogenase test can be relied upon for the exclusion of *C. difficile* carriage or infection and are ideally suited to screening large numbers of specimens, as the results are quickly available. Polymerase chain reaction (PCR) to detect toxin A (*tcd A*) or toxin B (*tcd B*) genes has sensitivity similar to cytotoxin testing. Real-time PCR tests that detect toxin A and B genes are highly sensitive and specific. The sensitivity of PCR is greater than EIA and comparable to cytotoxicity assay. Nucleic acid amplification tests (NAATs) based on the detection of toxin genes has become commercially available for the diagnosis of CDI. These methods have been compared to toxigenic culture (equivalent endpoint) and showed a good correlation. Results can be provided to clinicians within the same day as the stool sample. However, the best standard laboratory test for CDI diagnosis has not been clearly established. There are currently two reference assays available with different targets: the cytotoxicity assay that detects free toxins and the toxigenic culture which detects the organism with the potential to produce toxin. Only stools from patients with diarrhea should be tested for *C. difficile*.

**Keywords:** *Clostridium difficile* infection, colonoscopy, culture, EIA, NAAT, PCR,
Pseudomembranous colitis, recommendations

Full Text

*Clostridium difficile* infection (CDI) is a growing nosocomial and public health problem with mortality up to 25% in frail elderly people. CDI may present as fulminant colitis in approximately 3% of patients and account for most of the serious complications including perforation, prolonged ileus, megacolon and death. Accurate diagnosis of CDI is essential for optimal treatment and prevention but continues to be challenging.

(i) **Clinical diagnosis:** Clinically, the disease can be diagnosed by symptoms of explosive watery, green, foul-smelling or bloody diarrhea accompanied by abdominal cramps, leukocytosis, fever, nausea, anorexia, malaise, dehydration and delirium. Clinical suspicion for CDI is important because stool assays for diagnosing CDI are not widely available.

(ii) **Endoscopic diagnosis:** By inserting a flexible endoscope with a camera, the colon can be examined. Pseudomembranous colitis (PMC) when present appears as multiple yellow-white friable plaques, a few centimeters in size, attached to the underlying mucosa. Delay in the diagnosis of PMC could be lethal due to development of toxic megacolon (>7 cm diameter) accompanied by severe systemic toxicity or perforation. Endoscopy should be avoided in patients with paralytic ileus or colonic dilatation because of the risk of perforation. It is better reserved for special situations, such as when the patient is seriously ill and the results of rapid but not highly sensitive non-invasive tests are negative or delayed and CDI is strongly suspected.

(iii) **Computed tomographic scan/abdominal x-rays:** PMC can sometimes be diagnosed by computed tomographic (CT) scan when diarrhea is absent but abdominal pain, fever and leukocytosis occur. CT scan findings are most useful in PMC cases localized to the proximal colon and may reveal colonic distension, thickening, pericolonic inflammation, or free air. Abdominal plain films may also demonstrate small bowel dilatation, air-fluid levels and scalloping of the bowel wall due to submucosal edema.
(iv) **Conventional culture:** *C. difficile* grows on selective media providing a low cost method. The media generally contain antibiotics (cycloserin and cefoxitin) to ensure selectivity, and sometimes taurocholate or lysozyme to promote germination of spores and enhance sensitivity of the media. Chromogenic media are now available and allow an easy and more rapid identification of *C. difficile* due to the black color of the colonies. Ethanol or heat shock can be performed before plating in order to reduce the endogenous flora and optimize the recovery of *C. difficile* strains. Sensitivity is approximately 2000 bacteria/g of stool. But culture is dependent upon the presence of spores or viable vegetative cells. The procedure is cumbersome and requires several days for results. Moreover it requires a follow-up toxin testing as only about a third of the colonized isolates produce toxin. Culture nevertheless is useful for epidemiological and antibiogram studies during outbreaks.

(v) **Toxigenic culture:** Toxigenic culture is considered a gold standard for CDI diagnosis and has been approved by the US Food and Drug Administration. This is a two-step method based on the isolation of *C. difficile* in culture. The capacity of the strain to produce toxins *in vitro* is then determined by inoculating colonies in broth and testing the supernatant on cell culture or by enzyme immunoassays (EIAs) for toxins A and B performed directly on colonies. Polymerase chain reaction (PCR) targeting *tcdA* and/or *tcdB* after DNA extraction from colonies can also be performed in order to detect the presence of the genes encoding for toxins.

(vi) **Tissue culture:** *C. difficile* toxins can be detected in the fecal samples using tissue culture assay. Different cell lines can be used, but McCoy, MRC-5 and Vero cell lines are considered to be the most sensitive. It can detect as little as 1.0 pg of toxin B. The disadvantage of tissue culture is the difficulty in maintenance of cell cultures, Moreover the procedure is expensive and time-consuming. False negative results can occur in stored samples due to toxin degradation or by delay in transportation or by medication. In fact, a negative cytotoxicity assay does not completely rule out *C. difficile* as the cause of diarrhea as 30% of patients may be missed.

(vii) **Enzyme immunoassays:** EIA to detect toxin A or both toxins A and B in stool samples is widely used the world over. The common EIA employed have sensitivity of less than 50%. The advantage of EIA is predominantly the speed with which results are obtained, roughly two and a half hours. During unavoidable circumstances, stool specimens can be tested even if unrefrigerated for up to 13 h after collection. Glutamate dehydrogenase (GDH) test is as good
as culture in sensitivity and can help to exclude *C. difficile* carriage or infection and are ideally suited to screening large number of specimens.

**(viii) Molecular techniques:** PCR to detect toxin A or toxin B genes has sensitivity similar to cytotoxin testing. Only the US FDA–cleared qPCR assay can rapidly detect the *C. difficile* toxin B gene in a stool sample and is highly accurate. It is now being adapted by several laboratories and becoming more widely available. Real-time PCR tests that detect toxin A and B genes are highly sensitive and specific. The sensitivity of PCR is greater than EIA and comparable to cytotoxicity assay. In addition, PCR results can be available within as little as one hour. PCR can be used in an algorithm together with other assays such as EIA for GDH and EIA for toxins A and B. The PCR methods offer greater specificity, although their cost is greater and there is a risk that mutations in the toxin B gene may reduce their sensitivity in the future, which may go undetected if PCR is used alone.

A real-time cell analysis assay (ACEA Biosciences, CA, USA) based on electronic impedance technology was described in 2010 for quantitative detection of toxin B in stool samples. The system provides automated data acquisition in real-time and is amenable to a high-throughput, on-demand platform. However, this test is not routinely used in clinical laboratories.

In 2009, nucleic acid amplification tests (NAATs) based on the detection of toxin genes became commercially available for the diagnosis of CDI. These methods have been compared to toxigenic culture and showed a good correlation. Results can be provided to clinicians within the same day. According to the different assays, the tests are amenable to both batch and on-demand testing. The cost of these assays is still prohibitive for many laboratories and their place among the different diagnostic options remains to be clarified.

**Recommendations**

The best standard laboratory test for diagnosis has not been clearly established for the past 30 years. There are currently two reference assays for the diagnosis of CDI with different targets: the cytotoxicity assay that detects free toxins and the toxigenic culture which detects the organism with the potential to produce toxin.

a) NAAT for *C. difficile* toxin genes such as PCR are superior to toxins A+B enzyme
immunoassay testing as a standard diagnostic test for CDI.

b) GDH screening tests for *C. difficile* can be used with subsequent toxin A and B enzyme immunoassay testing but the sensitivity is lower than NAATs.

c) Only stools from patients with diarrhea should be tested for *C. difficile*.

d) Repeat testing should be discouraged.

e) Testing for cure should not be done.

References


Author’s Biography
Prof. Chetana Vaishnavi is a Medical Microbiologist working in the Department of Gastroenterology, Postgraduate Institute of Medical Education and Research, Chandigarh, India. She has actively participated in teaching and research for the past 34 years at the Institute. She is presently working in the area of gastrointestinal infections, particularly on *Clostridium difficile*, foodborne pathogens, biofilms in biliary stents, C-reactive protein, fecal lactoferrin, fecal myeloperoxidase etc. Prof. Vaishnavi has made significant contribution to medical science for which she has received several awards inclusive of ISG-Zydus Alidac Oration and the Dr CGS Iyer Oration by the ICMR. She is the Founder and Chairperson of Gastrointestinal Infection Society of India. She has produced and edited two multi-author books on ‘Infections of the Gastrointestinal System’ (Jaypee Publisher, 2013) and ‘Gastrointestinal Tract Infections’ (Paragon International Publisher, 2008). She did collaborative research work with national and international organizations. She is a Member of several Scientific Societies and has delivered invited lectures and chaired sessions in national and international conferences. She has published over 145 well-cited research papers and 19 chapters both nationally and internationally. She is a reviewer for several reputed National and International Journals and an examiner for several Indian Universities.

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