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Clostridium difficile Testing: Unified YNHH Algorithm

Clostridium difficile infection is a toxin-mediated disease. Toxigenic strains of *C. difficile* make two toxins, A and B. Contrary to prior understanding, it is **toxin B** not toxin A that is essential to disease (1,2). Available test methods detect different targets (*=gold standard tests):

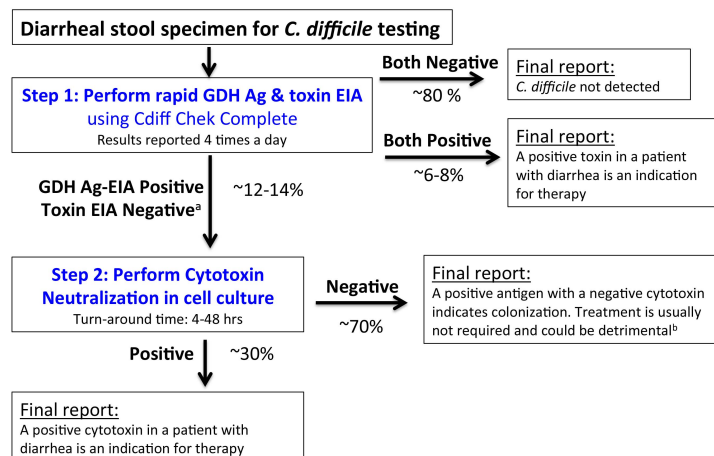
Target	Test Method	Time to result	Cost	Comments
<i>C. difficile</i> toxins A & B	Cytotoxin neutralization test*	4-48 hrs	+	Best indicator of disease
	Toxin enzyme immunoassay (EIA)	1-24 hrs	+	Indicates disease, but not sensitive
<i>C. difficile</i> bacteria (all)	Bacterial culture	2-3 days	+	Cannot differentiate toxigenic bacteria, or separate carrier from disease
	Bacterial GDH antigen EIA	1-24 hrs	+	
<i>C. difficile</i> toxigenic strains only	Toxigenic bacterial culture*	2-9 days	++	Detects more positives than toxin tests, but cannot separate toxigenic carrier from disease
	PCR (toxin B gene)	1.5-24 hrs	++ - +++	

Bacterial tests: GDH antigen tests economically and rapidly detect all *C. difficile* bacteria. PCR tests detect toxin B gene (i.e. toxigenic strains), but cannot determine whether toxin is being actively produced *in vivo*. It is increasingly recognized that even toxigenic *C. difficile* can colonize patients without causing disease (3-5). Treating asymptomatic carriers can have the adverse consequence of increasing subsequent disease by disrupting the balance between host and organism (4,5). Therefore treating carriers is not currently recommended. A large multicenter, prospective study has shown that **morbidity and mortality** correlate with **cytotoxin positivity** and **not PCR**; notably GeneXpert PCR was not recommended as a single test due to its low positive predictive value (6). Other studies, including two at YNHH, raised similar concerns about PCR (7-9).

Toxin tests: Differentiating patients with disease requiring treatment from colonized patients who have diarrhea due to other causes relies on detection of toxin in diarrheal stool. YNHH is unusual in that cytotoxin testing is still available for clinical diagnosis, with cell culture plates prepared on site in the virology laboratory. The "cytotoxin neutralization test" is a biological assay in which the toxin disrupts cells in culture (as it does to cells in the gut), and the toxic effects are then neutralized by specific *C. difficile* anti-toxin. In contrast, toxin A+B EIA tests are simple, fast, but lack sensitivity. Therefore if used, toxin EIA tests should be supplemented by cytotoxin assays.

YNHH test algorithm: In January 2015, the two campuses of YNHH (York Street and St. Raphael) will have a unified *C. difficile* test algorithm (see below). All testing will be done at York Street laboratories. A rapid GDH antigen/toxin EIA test will promptly identify negatives and most positives, followed by cytotoxin test when needed to confirm *C. difficile* disease. For colonized patients with significant diarrhea for other reasons, it is important to prevent transmission to others by contact isolation and rigorous environmental cleaning. PCR will be available upon request for infection control purposes (GDH antigen positive, cytotoxin negative, with continued diarrhea) and for other difficult cases. At present, an Infection Control, ID or GI specialist must contact the lab to request PCR. Stools will be held for up to 3-6 days.

Sample submission: Submit only diarrheal stools, and no more than one stool per 3 day period if cytotoxin is negative. Do not retest for 14 days if cytotoxin-positive, if on treatment, or for test of cure, since positive lab results can persist despite effective therapy (1).



a. Rapid toxin assays detect only 60-80% of cytotoxin positives.

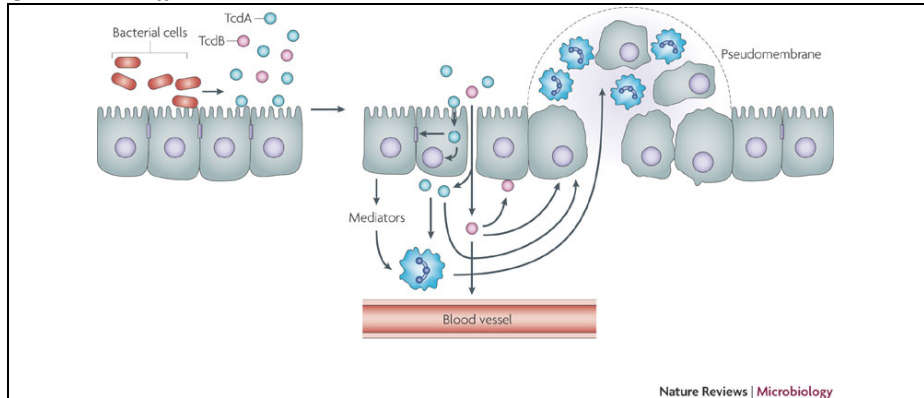
b. If diarrhea persists, PCR for toxin genes should be ordered on Ag-positive/cytotoxin-negative patients by Infection Control to determine the need for continued contact isolation. Stools will be held for up to 3-6 days in the laboratory.

APPENDIX

I. **Clinical manifestations:** *C. difficile* can cause a spectrum of symptoms, however most diarrhea in hospitalized patients is **not** due to *C. difficile*.

Disease spectrum	Diagnosis of <i>C. difficile</i> infection (i.e. disease)
Asymptomatic Diarrhea Colitis Pseudomembranous colitis Fulminant colitis (e.g. ileus, toxin megacolon)	1. Combination of signs and symptoms, confirmed by lab tests, in absence of other cause 2. Colonoscopic or histopathologic evidence of pseudomembranous colitis

II. Pathogenesis of *C. difficile* toxin-mediated disease



(Ref: Rupnik M et al, Nat Rev Microbiol; 2009; 7:526-36.)

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