Yale NewHaven Health
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Clostridium difficile Test Algorithm: Rationale, Methods and Results

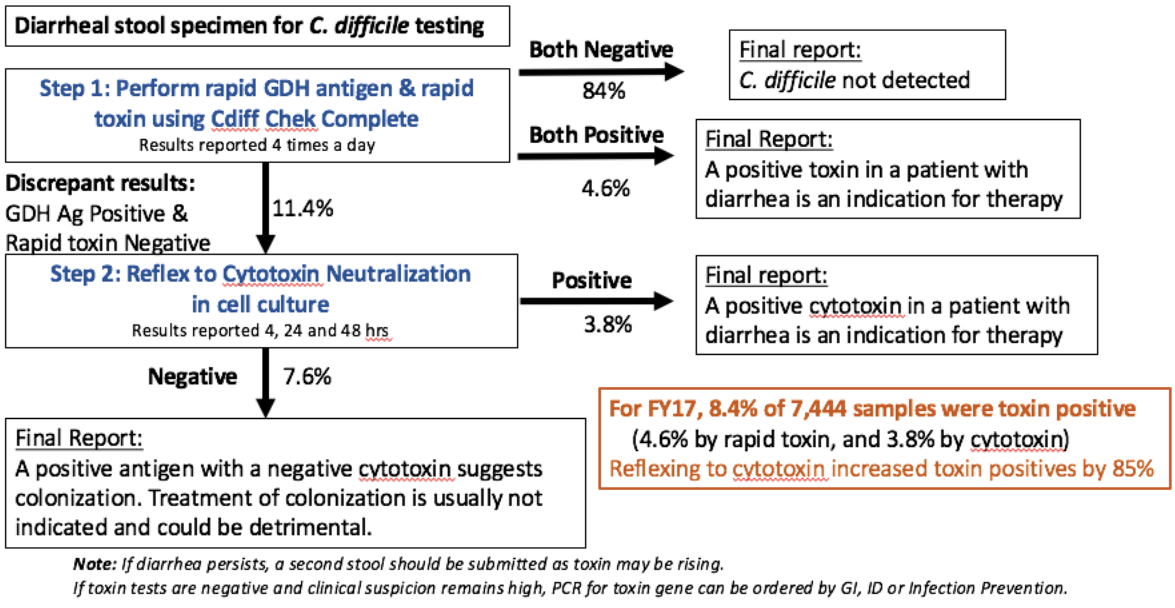
Since 2015, the *Clostridium difficile* Performance Improvement Team has spearheaded the effort to reduce *C. difficile* disease at YNHH. This has led to a dramatic 45% decrease in cases, from 9.01 to 4.95 per 10,000 patient days. Key elements of the *C. difficile* "bundle" included reducing the use of fluoroquinolones, unnecessary antibiotics, and proton pump inhibitors that drive disease, prompt and strict isolation, and stringent environmental cleaning to prevent transmission. The Laboratory assists this effort by providing prompt and accurate diagnostic test results. YNHH utilizes a unique 2-step algorithm to diagnose *C. difficile* disease, and this can lead to confusion. In this newsletter, the rationale and results for the test algorithm are presented, as well as images of the methods (**Appendix page 2**.)

Rationale: *C. difficile* infection is a toxin-mediated disease. Toxigenic strains of *C. difficile* make two toxins, A and B. Contrary to our initial understanding, Toxin B rather than toxin A is essential for disease (1,2). PCR tests determine whether a *C. difficile* strain carries the toxin B gene, but PCR does not determine whether the toxin is being actively produced *in vivo*. Several studies have shown that PCR tests over-diagnosed *C. difficile* disease and that morbidity and mortality correlated with detection of cytotoxin in stool (3-5). At YNHH, we also found that disease correlated better with cytotoxin than PCR alone (6-7). It is now recognized that toxigenic strains of *C. difficile* can colonize patients without causing disease, and that treating asymptomatic carriers can have adverse consequences (8-10).

YNHH is unusual in that **cytotoxin testing**, a diagnostic gold standard, is still available for clinical diagnosis, using cell culture plates prepared on site in the Virology Laboratory. The **cytotoxin neutralization test** is a biological assay in which the toxin damages the cells in culture, as it damages cells in the gut. To confirm specificity, the toxic effects must then be neutralized by *C. difficile* antitoxin. The downside is that cytotoxin results are read microscopically at **4, 24 and 48 hours**. In contrast, rapid toxin immunoassays are simple and fast, but lack sensitivity. The YNHH 2-step algorithm combines the speed of the immunoassays with the sensitivity of cytotoxin.

YNHH Test Algorithm: A rapid *C. difficile* GDH bacterial antigen/ toxin EIA test is performed 4 times a day in the Virology Laboratory and takes 30 minutes to complete. In FY17, **88.6%** of samples had a **final result in Step 1**: both GDH antigen and toxin negative (84%) or both positive (4.6%). If discrepant results are obtained, i.e. GDH antigen positive, but rapid toxin negative, the stool is reflexed in **Step 2** to the cytotoxin test. About 1/3 of reflexed samples are cytotoxin positive. In FY17, reflexing to cytotoxin for GDH positive samples increased the number of **toxin-positive patients** identified by 85%, from 4.6% to **8.4% of stool samples submitted**.

Algorithm at YNHH and Results from Oct 2016-Sept 2017



Note: *If diarrhea persists, a second stool should be submitted as toxin may be rising.*
If toxin tests are negative and clinical suspicion remains high, PCR for toxin gene can be ordered by GI, ID or Infection Prevention.

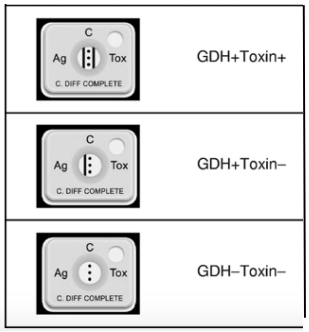
***C. difficile* toxin gene PCR results:**
No test is 100% sensitive. Therefore, Toxin gene PCR is available if antigen and/or cytotoxin tests are negative and clinical suspicion remains high. PCR can be ordered by an ID, GI or Infection Prevention attending by calling the Virology Laboratory at 688-3524.

In FY17, PCR was performed, on request, on only two patients and both were negative for *C. difficile* toxin B gene by PCR.

Sample submission: Liquid or semisolid stools (that conform to the contour of the container) should be submitted in leakproof containers. Solid stools will be rejected. If tests are negative, repeat testing can be ordered at 3 days. Tests for cure should not be done, as patients can remain positive despite successful therapy.

Testing is done primarily in Virology due to the expertise in cytotoxin neutralization, with Microbiology backup when Virology is closed and for *C. difficile* PCR. Routine questions should be referred to the Virology Laboratory.

Appendix:
Test Methods:
1. **Main screening test: C. Diff Chek Complete rapid immunoassay** (done 4 times a day, ~30 minutes to result)



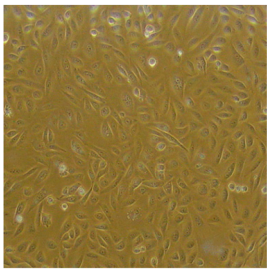
Positive for both bacterial GDH antigen and toxin

C. diff bacteria present (GDH Ag+): **Reflex to cytotoxin**

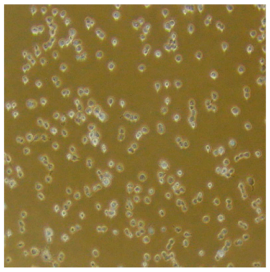
Negative for both bacterial GDH antigen and toxin

2. **Reflex GDH+Toxin-**samples to **C. diff Cytotoxin in cell culture** (biologic assay read microscopically at 4, 24 and 48 hrs)

Normal cell culture monolayer



C. difficile cytotoxin effect on cells



3. **Xpert® *C. difficile* PCR:** Available on request by ID, GI, or Infection Prevention. PCR is performed in Microbiology.

Prepared by Marie L. Landry, M.D., Director Clinical Virology Laboratory.
For questions or concerns, contact the Clinical Virology Laboratory at 203-688-3524.

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