Yale NewHaven Health
Yale New Haven Hospital

Laboratory Updates: Clinical Virology and Microbiology

Vol. 33 (1) **May 2024**

Clostridioides difficile: YNHHS Test Algorithm Change and Update

C. difficile* infection (CDI) is a toxin-mediated disease** and an important hospital-acquired infection (HAI). Toxigenic strains of *C. difficile* can produce **toxins that damage the gut mucosa**. The mere presence of toxigenic bacteria does not confirm CDI, as **colonization is common**, as is diarrhea from non-CDI causes. Use of PCR testing alone can lead to over-diagnosis which in turn can result in unnecessary therapy, multi-drug resistance, and higher risk of CDI in the future. Thus, detection of **toxin production *in vivo should be used to guide therapy. The previous YNHH algorithm screened for both *C. difficile* GDH bacterial antigen and free toxin in stool by immunoassay, with a reflex to a cytotoxin cell culture assay, to increase sensitivity of toxin detection when GDH antigen was positive, but toxin immunoassay was negative.

The **cytotoxin cell culture assay** detects toxin biologic activity in cell culture and can detect low levels of toxin missed by toxin immunoassays. However, due to the expertise required and the long time to results, its use is usually limited to research studies. Since cell culture expertise was available in the Yale Virology Laboratory, the **cytotoxin assay was performed for clinical use at Yale until 2023** and was notably more sensitive than similar assays performed at reference labs. However, when cell culture for virus isolation was discontinued in 2022, it became no longer feasible to maintain the cytotoxin cell culture assay. In addition, the greater sensitivity of the cytotoxin cell culture assay led to a higher CDI rate for YNHHS when compared to facilities that detected toxin by immunoassay only. This higher detection rate contributed to financial penalties by CMS and a lower Leapfrog group safety rating for YNHHS hospitals.

On July 19, 2023, working with the Diagnostic Stewardship Committee under Dr. Deborah Rhodes, YNHHS converted to a testing algorithm used successfully by many of our peers: screening for toxigenic strains by ***C. difficile* toxin B gene PCR**, with reflex to rapid **toxin immunoassay** if PCR is positive. This new algorithm has **shortened the turnaround time (TAT) for all results to 2-4 hours**, facilitated **isolation of colonized patients with diarrhea** to prevent nosocomial transmission, and alerted clinicians to **avoid high-risk antibiotics** if feasible. The new algorithm test interpretation guide and flow chart are shown on **page 2**. Clinicians are also advised to only test patients with high pre-test probability for CDI and to use the **C. diff Care Signature Pathway** and decision support tools in EPIC.

Ensuring patient safety: Impact of discontinuing the cytotoxin cell culture assay on patient outcomes

Due to concern about the patient impact of no longer offering the cytotoxin cell culture assay, PCR-positive stools were saved for several months and then tested off-line by the cytotoxin assay. Of 278 stools saved and tested, **81 were negative by toxin immunoassay but positive by cytotoxin cell culture assay**. Notably, median cytotoxin titers were 100-fold lower when the toxin immunoassay was negative. Chart reviews were performed on these 81 patients to assess treatment and outcomes. A summary is below.

Chart review for toxin immunoassay-negative, cytotoxin cell culture-positive stools (n=81)

Not treated	Outcomes of untreated patients	Treated	Reasons in chart for treatment of patients
n=68 (84%)	**Subsequent CDI:** -Two patients improved without therapy, then had recurrent diarrhea **12 and 60 days later.** Stools were then found to be **rapid toxin positive** and patients were treated for CDI. **Other CDI complications**: None recorded	**n=13 (16%)**	-Two patients were on therapy when tested, potentially giving rise to false-negative toxin. -One patient treated for CDI had diarrhea recur when treatment was stopped and was retreated. -Positive PCR result (misinterpreted?). -Severe diarrhea. -Patient fragile or critically ill. -Prior history of CDI.

Summary

1. **Stools positive only by the cytotoxin cell culture assay had median 100-fold lower cytotoxin titers** in cell culture when compared to stools positive by both toxin immunoassay and cytotoxin cell culture assay. [No stools were positive by toxin immunoassay only.]

2. **Adverse patient outcomes were not identified** in our study for the **cytotoxin-only-positive untreated** patients.
3. These results suggest that discontinuing the high sensitivity cytotoxin cell culture assay at Yale has not adversely impacted patient outcomes, and that patients with low levels of cytotoxin appear to do well without therapy.
4. For patients with significant, worsening diarrhea, clinicians should **retest** (bypassing the EPIC block if needed) to exclude rising toxin levels; consider **endoscopy** if toxin is repeatedly negative; or consider empiric therapy if fulminant disease, patient is critically ill with significant diarrhea without other explanation and CDI is most likely etiology.
5. The **new test algorithm has many benefits**: shortened TAT, isolation of carriers with diarrhea, potential reduction in high-risk antibiotic use, and as a consequence of these enhancements, hopefully reduced *C. difficile* HAI.
6. Going forward, the transition to the widely used algorithm of **PCR with reflex to toxin immunoassay should allow a fair comparison of CDI rates** by CMS/NHSN between YNHHS and other institutions using the same algorithm.

C. difficile Testing Algorithm & Interpretation

Endorsed by Diagnostic Stewardship, Diarrhea Clinical Consensus Group and System Antimicrobial Stewardship Effective July 19, 2023

Result	Screening Test: C. diff PCR	Toxin Test: C. diff Toxin EIA	Treatment Indicated	Interpretation	Repeat Testing	Isolation
C. difficile **Not detected**	*C. difficile* PCR: **NEGATIVE**	Toxin: **Not done; not indicated**	NO	Toxigenic *C. difficile* DNA was **NOT detected.** Alternative causes of diarrhea should be considered. Empiric treatment is not indicated unless there is strong clinical evidence of *C. difficile* disease (e.g., pseudomembranous colitis, toxic megacolon).	Repeat testing within 7 days is not indicated unless there is a major clinical change or high clinical suspicion.	**NO**
C. difficile **Colonization suggested**	*C. difficile* PCR: **POSITIVE**	Toxin: **NEGATIVE**	NO	Suggests COLONIZATION; treatment is NOT generally recommended. A **POSITIVE** *C. difficile* PCR with a **NEGATIVE** toxin suggests COLONIZATION. Alternative causes of diarrhea should be considered. Empiric treatment is not usually indicated unless fulminant disease is suspected (e.g., toxic megacolon).[1,2] If possible, avoid the use of high-risk antibiotics associated with CDI (e.g., clindamycin, fluoroquinolones, 3rd/4th generation cephalosporins) in colonized patients.[3,4]	Repeat testing within 7 days is not indicated unless there is a major clinical change or high clinical suspicion.	**YES** For colonization: Contact Plus
C. difficile Infection **Confirmed**	*C. difficile* PCR: **POSITIVE**	Toxin: **POSITIVE**	YES	A **POSITIVE** *C. difficile* PCR with a **POSITIVE** toxin in a patient with diarrhea is an indication for therapy.	Follow-up testing for cure is not recommended. Repeat testing is only indicated if the patient has a new onset of symptoms after 14 days of therapy.	**YES** For active infection: Contact Plus

This new testing strategy will be performed on all days and shifts at all campuses and will accelerate result reporting relative to the previous testing strategy.
*The Infectious Disease Service and/or Antimicrobial Stewardship Team is available for consult as needed.

1. Polage et al., *JAMA* IM 2015
2. Planche et al., *Lancet* ID 2013
3. Brown KA et al., *CID* 2021
4. Brown KA et al., *AAC* 2013

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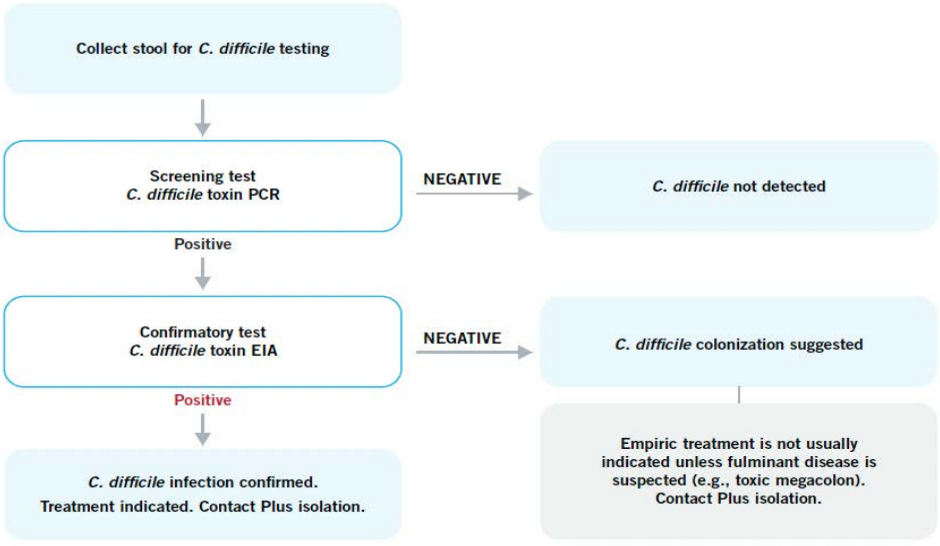

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Testing of neonates and infants: Although neonates and infants frequently are colonized with toxigenic *C. difficile*, they rarely develop symptomatic disease. High levels of *C. difficile* organisms and toxins (levels similar to those in adults with pseudomembranous colitis) can be found in the stools of healthy, asymptomatic neonates and infants.

Therefore, to avoid falsely attributing symptoms to CDI, routine testing of children <2 years of age is blocked in EPIC and recommended only in specific circumstances when other causes of diarrhea have been excluded. **Please call the Lab to bypass the block if criteria are met**. [https://www.uptodate.com/contents/clostridioides-difficile-infection-in-children-clinical-features-and-diagnosis?search=c%20difficile%20children&topicRef=6041&source=see_link].

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8. Shim JK, Johnson S, Samore MH, Bliss DZ, Gerding DN. 1998. Primary symptomless colonisation by *Clostridium difficile* and decreased risk of subsequent diarrhoea. Lancet 351:633---636.
9. Kyne L, Warny M, Qamar A, Kelly CP. 2000. Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. N Engl J Med342:390---397.
10. Turner NA, Krishnan J, Nelson A, Polage CR, Cochran RL, Fike L, Kuhar DT, Kutty PK, Snyder RL, Anderson DJ. Assessing the Impact of 2-Step *Clostridioides difficile* Testing at the Healthcare Facility Level. Clin Infect Dis. 2023 Oct 5;77(7):1043-1049. doi: 10.1093/cid/ciad334. PMID: 37279965; PMCID: PMC10552580.
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