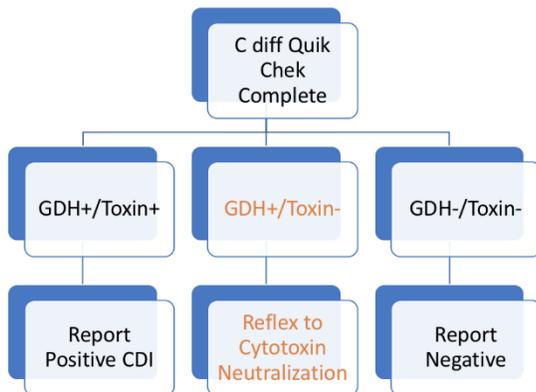


Standardization of *Clostridium difficile* Testing across YNHHS

C. difficile infection (CDI) is a **toxin-mediated disease** and an important hospital associated infection (1). However, laboratory diagnosis is not standardized and algorithms vary. *C. difficile* toxin immunoassays have suboptimal sensitivity and may under-diagnose CDI, whereas PCRs that detect the toxin gene can detect carriers of toxigenic strains of *C. difficile* as well as true cases, and thus can over-diagnose CDI (2). **Cytotoxin neutralization in cell culture** is a biologic assay that is more sensitive than toxin immunoassays and has been shown to best correlate with CDI; however, it is rarely available for clinical use (3). The **cytotoxin test** is still available for clinical diagnosis in the Yale New Haven Hospital Virology Laboratory and access to this test is being extended to all YNHHS hospitals.

As part of the *C. difficile* Care Signature Pathway, the laboratory diagnosis of *C. difficile* infection (CDI) will be harmonized across YNHHS. **C. DIFF QUIK CHEK COMPLETE immunoassay** is used across YNHHS as the initial screen to rapidly detect both *C. difficile* bacterial antigen (GDH) and toxin. When GDH and toxin are both positive or both negative, final results are reported. **When results of GDH and toxin are discordant, a second test is recommended.**



In New Haven, all discordant samples have been reflexed to cytotoxin neutralization in the Clinical Virology Laboratory.

Of these **discordant** samples, 28-32% are cytotoxin positive and are diagnosed as CDI.

At other system hospitals, PCR is available as a second step, but is only performed at provider discretion due to concern for PCR overdiagnosis. However, without a second step, CDI cases may be missed.

To promote accurate detection of CDI cases, **all samples with discordant C DIFF QUIK CHEK COMPLETE results will be sent to New Haven for cytotoxin neutralization. Final Cytotoxin results are reported in 24-48 hours of receipt at Yale New Haven Hospital Virology.** Interpretations of results that will display in Epic are given below. **See screenshots in Appendix.**

GDH	Toxin	Cytotoxin	Result interpretation in Epic
NEG	NEG	Not done	False negative results can occur early in disease or with very dilute or poor samples (e.g. mixed with urine). If suspicion is high, or patient is high risk and diarrhea persists, submit a second sample (call (203) 688-2444 if needed). If there is a strong pre-test probability of CDI, obtain a gastroenterology consult and consider empiric therapy. For difficult cases, the cytotoxin assay and/or PCR can be obtained by calling the laboratory. GDH antigen screen has >95% sensitivity for detection of <i>C. difficile</i> bacteria, with a negative predictive value of ~98%.
POS	POS	Not done	A positive toxin in a patient with diarrhea is an indication for therapy.
POS	See cytotoxin	Pending	The rapid toxin test was negative but is only 60% sensitive. Thus, the more sensitive cytotoxin neutralization test is being done, with results reviewed after 4, 24, and 48 hours of incubation. Positive results are reported when first observed. See Cytotoxin result to determine need for therapy.
		Positive (FINAL)	A positive <i>C. difficile</i> cytotoxin in a patient with diarrhea is an indication for therapy. Effectiveness is determined by the clinical response, not repeat testing for toxin. Positive toxin results can persist for days to weeks after successful treatment.
		Negative (FINAL)	A positive bacterial antigen with a negative cytotoxin indicates colonization. Treatment of colonization is usually not indicated and could be detrimental. However, if symptoms worsen, a second stool sample should be tested, as toxin may be rising.

If questions, clinicians can call their hospital laboratory. This change will be implemented November 1, 2021.

Additional Ordering Guidance:

1) There is no change in the *C. difficile* assay order. 2) However, the Care Signature Pathway must be used to place the order. 3) The Lab will reflex discordant samples; the clinician does not need to take additional action. 4) Contact Plus Isolation Precautions should NOT be ordered at the same time as *C. diff* testing unless suspicion for CDI is very high. 5) Order Contact Plus Precautions if C. DIFF QUIK CHEK COMPLETE result is POSITIVE for both antigen and toxin, is or discordant reflexing to cytotoxin.

Sample submission: Liquid or semisolid stools that conform to the container should be submitted in leakproof containers (Bristol 6 or 7). Solid stools will be rejected. Tests for cure should not be done, as patients can remain positive despite successful therapy.

PCR can be done on request by IP, ID, GI for cytotoxin-negative samples: No test is 100% accurate and thus PCR can still be performed for difficult cases if needed, as requested by Infection Prevention, Infectious Disease or GI services. Please call the laboratory. *Note: A positive PCR result confirms the presence of toxigenic C. difficile but may still represent colonization and not CDI.*

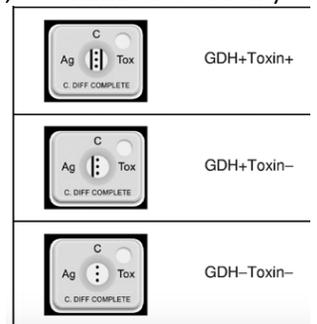
Test Methods:

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



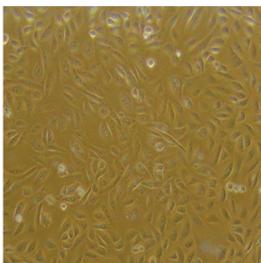
Positive for both bacterial
GDH antigen and toxin

C. diff bacteria present
(GDH Ag+): **Reflex to
cytotoxin**
Negative for both bacterial
GDH antigen and toxin

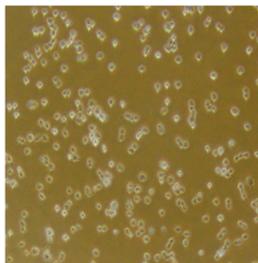


2. **Reflex GDH+Toxin-** samples to ***C. diff* Cytotoxin in cell culture** (biologic assay read microscopically at 4, 24, 48 hrs)

Normal cell culture monolayer



C. difficile cytotoxin effect on cells



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

Newsletter prepared by Marie L. Landry, M.D. YNHH Clinical Virology Laboratory.

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APPENDIX: Epic SCREEN SHOTS FINAL RESULTS

1. CONCORDANT: GDH and Rapid Toxin Negative → **Negative for C. diff**

10/26/2021 1950	
SPECIAL INFECTIOUS...	
Treponema pallidum...	
C. difficile GDH A...	<i>Negative</i>
C. difficile Rapid...	<i>Negative *</i>

2. CONCORDANT: GDH and Rapid Toxin Positive → **Positive for C. diff**

10/26/2021 0453	
SPECIAL INFECTIOUS...	
C. difficile GDH A...	<i>Positive</i> !
C. difficile Rapid...	<i>Positive *</i> !

3. DISCORDANT (GDH+, Rapid toxin-), Reflex to Cytotoxin. Final result: Cytotoxin Negative → **Negative for C. diff**

10/23/2021 2358	
SPECIAL INFECTIOUS...	
C. difficile GDH A...	<i>Positive</i> !
C. difficile Rapid...	<i>See Cytotoxin ... *</i>
C. difficile Cytot...	<i>Negative *</i>

4. DISCORDANT (GDH+, Rapid toxin-), Reflex to Cytotoxin. Final result: Cytotoxin Positive → **Positive for C. diff**

10/24/2021 1423	
SPECIAL INFECTIOUS...	
C. difficile Antig...	
C. difficile GDH A...	<i>Positive</i> !
C. difficile Rapid...	<i>See Cytotoxin ... *</i>
C. difficile Cytot...	<i>Positive *</i> !