Legacy Laboratory Services

Clostridium difficile Infection Detection by Polymerase Chain Reaction (PCR)

July 2010

New testing method provides faster and more sensitive test for *Clostridium difficile* infection.

C. difficile assay by PCR has recently been added to our test menu. The Cepheid Xpert assay is an automated DNA test for the qualitative detection of the toxin B gene (*tcd*B) of *Clostridum difficile,* from unformed stool specimens of patients suspected of having *Clostridium difficile* infection (CDI). This test utilizes real-time polymerase chain reaction (PCR) methodology and offers increased sensitivity and improved turn-around time over other methods[5].

The Xpert *C. diff* PCR assay replaces the cell culture cytotoxicity neutralization assay (CCCN), previously performed as the confirmatory method for CD Toxin (*C difficile* toxin test). The two-step algorithm (see attached) begins with a glutamate dehydrogenase (GDH) EIA assay performed on unformed stool, to detect the presence/absence of *C. difficile,* regardless of its toxigenic capability. If GDH-positive, the specimen proceeds to a confirmatory *C. difficile* toxin test, which is now the Xpert *C. difficile* PCR assay. Because the PCR is completed within a matter of hours, unlike CCCN, which required up to two days, most CD Toxin orders will now be turned around within 24 hours. Screening for GDH before confirmation of positives by PCR is cheaper than screening all specimens by PCR and is an effective method for routine use[6]. Improved turn-around times help optimize patient care and infection control measures. **NOTE**: The Xpert *C. diff* PCR assay may be ordered as a stand-alone test (***C. difficile* toxin by PCR**), bypassing the GDH EIA[2].

C. difficile continues to challenge clinicians, epidemiologists, and microbiologists and CDI has increased substantially in the last decade. It is the cause of 15-25% of cases of antibiotic-associated diarrhea and 95-100% of cases of antibiotic-associated pseudomembranous colitis. Moreover, the emergence of severe and fatal disease among otherwise healthy persons with minimal risk factors, and community acquisition has now occurred. Rapid, accurate reporting of *C. difficile* is essential for improving patient outcomes and minimizing healthcare-associated infections[3].

According to the recent SHEA guidelines[4], testing for *C. difficile* or its toxins should only be performed on diarrheal (unformed) stools, unless ileus is suspected. A single specimen at the onset of a symptomatic episode is usually sufficient and routine testing of multiple specimens is NOT supported. Testing on asymptomatic patients is not clinically useful, including use as a test of cure. Specimens that are formed or hard will be rejected, and rectal swabs and fluid obtained at colonoscopy are not acceptable and will be rejected.

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This test is only approved for patients over two years of age. Children less than two years will have the two step algorithm performed, with the second step being CCCN. *C difficile* testing is of questionable utility in young children as the rates of symptomatic carriage (coexisting diarrhea) are not dissimilar to rates for asymptomatic carriage (15% to 63% of neonates, 3% to 33% of infants and toddlers younger than two years of age.

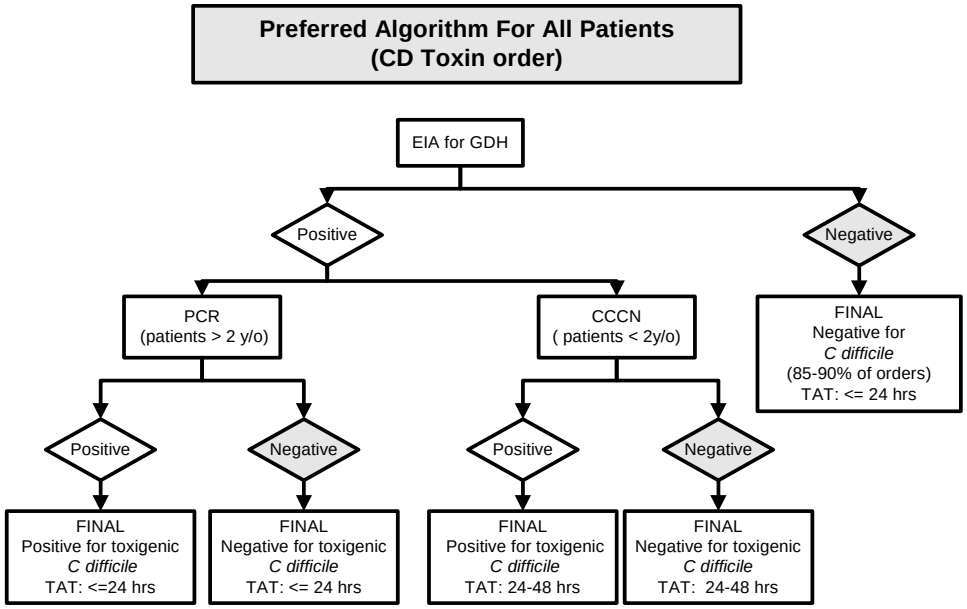
Collection guidelines

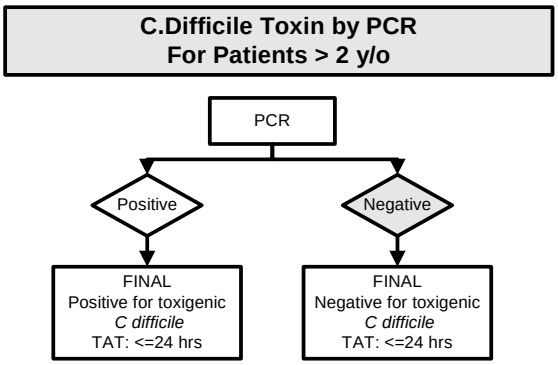
Test Name	**Clostridium difficile toxin by PCR**	**C difficile toxin detection**
Specimen	Fresh unformed stool (soft or liquid) in a leak proof container or Enteric Transport	Fresh unformed stool (soft or liquid) in a leak proof container or Enteric Transport. DO NOT FREEZE
Volume	Preferred is 5 mL, minimum is 2 mL	Minimum 2 mL
Transport	Refrigerated	Refrigerated
Rejection Criteria	Specimens past stability or stool in any preservative including SAF, PVA, or formalin. Formed stool, rectal swabs or colonoscopy fluid, unless approved by Microbiology. Duplicate testing unless approved by Microbiology.	Specimens past stability or stool in any preservative including SAF, PVA, or formalin. Formed stool, rectal swabs or colonoscopy fluid, unless approved by Microbiology. Duplicate testing unless approved by Microbiology.
Stability	24 hours room temperature, up to 3 days refrigerated (2-8°C)	24 hours room temperature, up to three days refrigerated (2-8°C)
Test Performed	Sunday - Saturday, 0700-1500	Sunday - Saturday, 0700-1500
Turnaround	24 hours	24 hours
Method	Real-time reverse transcription PCR	EIA for GDH, followed by PCR if GDH positive
Reference Values	Negative for toxigenic *C difficile*	Negative for toxigenic *C difficile*

References:

1) Novak-Weekly, S. et al, "*Clostridium difficile* Testing in the Clinical Laboratory by Use of Multiple Testing Algorithms". Journal of Clinical Microbiology, March 2010, 48:889-93.
2) Haihui Huang, et al, "Comparison of a Commercial Multiplex Real-Time PCR to the Cell Cytotoxicity Neutralization Assay for Diagnosis of *Clostridium difficile* Infections." Journal of Clinical Microbiology, November 2009, 47:3729-3731.
3) Nolte, Fredrick "Molecular Testing for *C difficile* at Medical Unversity of South Carolina: Validation, Implementation, and Realized Benefits." BD Inform Educational Seminar, March 2010.
4) SHEA-IDSA Guideline: Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). March 2010.
5) Package Insert, Xpert C Difficile. 2009. Cepheid, Sunnyvale, CA.
6) Goldenberg SD et al, "Two-Step Glutamate Dehydrogenase Antigen Real-Time Polymerase Chain Reaction Assay for Detection of Toxigenic *Clostridium difficile*." J Hosp Infect. Jan 2010; 74(1): 48-54.
7) "Clostridium difficile – pathogen or pest?" Paediatric & Child Health 2000; 5(6): 349-52

Clostridium difficile Testing Algorithms





Definitions:
PCR = Polymerase Chain Reaction
CCCN = Cell Culture Cytotoxicity Neutralization Assay
GDH = Glutamate Dehydrogenase
EIA = Enzyme Immunoassay

NOTE: Novak-Weekley[1] found the GDH + Xpert algorithm, as compared against *C. difficile* toxigenic culture (toxigenic culture now reaccepted/replacing CCCN as the true gold standard, but requiring too much time to be clinically useful), yielded sensitivity, specificity, positive predictive, and negative predictive values of 86.1, 97.8, 88.6 and 97.2%. Alternatively, an algorithm employing GDH + toxin EIA + CCCN (if toxin EIA was negative) yielded corresponding values of only 83.1, 96.7, 83.1 and 96.1%, and on average required 2 days to complete.

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