

## Overview

### Useful For

Rapid diagnosis of *Clostridioides difficile*-associated diarrhea (CDAD) and pseudomembranous colitis (PMC)

### Testing Algorithm

See [Laboratory Testing for Infectious Causes of Diarrhea](#) in Special Instructions.

### Special Instructions

- [Infectious Specimen Shipping Guidelines](#)
- [Laboratory Testing for Infectious Causes of Diarrhea](#)

### Method Name

Real-Time Polymerase Chain Reaction (PCR) using Fluorescent Resonance Energy Transfer (FRET)

### NY State Available

Yes

## Specimen

### Specimen Type

Fecal

### Ordering Guidance

This test is validated for formed feces, although testing formed feces for *Clostridioides difficile* is generally not clinically indicated.

### Shipping Instructions

See [Infectious Specimen Shipping Guidelines](#) in Special Instructions for shipping information.

### Necessary Information

**Specimen source is required.**

### Specimen Required

The high sensitivity of amplification by polymerase chain reaction requires the specimen to be processed in an environment in which contamination of the specimen by *Clostridioides difficile* toxin DNA is unlikely.

**Submit only 1 of the following specimens:**

**Preferred:**

**Specimen Type:** Preserved feces

**Supplies:** C and S Vial (T058)

**Container/Tube:** Commercially available transport system specific for recovery of enteric pathogens from fecal specimens (15 mL of nonnutritive transport medium containing phenol red as a pH indicator, either Cary-Blair or Para-Pak C and S)

**Specimen Volume:** Representative portion of feces; 5 mL

**Collection Instructions:**

1. Collect fresh fecal specimen and submit in container with transport medium.
2. Place feces in preservative within 2 hours of collection.

**Specimen Stability Information:** Ambient (preferred) <7 days/Refrigerated <7 days

**Acceptable:**

**Specimen Type:** Unpreserved feces

**Supplies:**

Stool container, Small (Random), 4 oz Random (T288)

Stool Collection Kit, Random (T635)

**Container/Tube:** Fecal container

**Specimen Volume:** Representative portion of feces

**Collection Instructions:** Collect fresh fecal specimen and submit representative sample in fecal container.

**Specimen Stability Information:** Refrigerated (preferred) <7 days/Frozen <7 days

**Forms**

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

-[Microbiology Test Request](#) (T244)

-[Gastroenterology and Hepatology Client Test Request](#) (T728)

**Specimen Minimum Volume**

1 mL

**Reject Due To**

Feces in gel transport medium ECOFIX preservative Formalin or polyvinyl acetate (PVA) fixative Preserved feces received frozen	Reject
---	--------

**Specimen Stability Information**

Specimen Type	Temperature	Time	Special Container
Fecal	Varies	7 days	

## Clinical and Interpretive

### Clinical Information

*Clostridioides difficile* (formerly *Clostridium difficile*) is the cause of *C difficile*-associated diarrhea (CDAD), an antibiotic-associated diarrhea, and pseudomembranous colitis (PMC). In these disorders bacterial overgrowth of *C difficile* develops in the colon, typically as a consequence of antibiotic usage. Clindamycin and broad-spectrum cephalosporins have most frequently been associated with CDAD and PMC, but almost all antimicrobials may be responsible. Disease is related to production of toxin A and B. Treatment typically involves withdrawal of the associated antimicrobials and, if symptoms persist, orally administered and intraluminally active metronidazole, vancomycin, or fidaxomicin. Intravenous metronidazole may be used if an oral agent cannot be administered. In recent years, a more severe form of CDAD with increased morbidity and mortality has been recognized as being caused by an epidemic toxin-hyperproducing strain of *C difficile* (NAP1 strain). Many toxin-hyperproducing isolates also contain the binary toxin gene and are resistant to quinolones. This test does not differentiate between toxin-hyperproducing and nontoxin-hyperproducing strains.

Traditionally, diagnosis relied upon:

1. Clinical and epidemiologic features
2. Culture, which is labor intensive and time consuming
3. Cytotoxicity assays, which are also labor intensive and time consuming
4. Toxin detection immunoassays, which are insensitive

The described polymerase chain reaction assay detects the regulatory gene (*tcdC*) responsible for production of toxins A and B. This test is used for rapid diagnosis of CDAD and PMC enabling prompt treatment that may reduce hospital stays for inpatients with CDAD.

### Reference Values

Not applicable

### Interpretation

A positive polymerase chain reaction (PCR) result for the presence of the gene regulating toxin production (*tcdC*) indicates the presence of *Clostridioides difficile* and toxin A and/or B.

A negative result indicates the absence of detectable *C difficile tcdC* DNA, but does not rule-out *C difficile* infection and may occur due to inhibition of PCR, sequence variability underlying primers or probes, or the presence of *C difficile* DNA in quantities less than the limit of detection of the assay.

### Cautions

The assay must be performed on fresh feces, fresh-frozen feces, or feces in transport medium.

The assay has not been validated as a test of cure. Since nucleic acid may persist after effective treatment, follow-up testing of a positive result is not recommended.

Interfering substances in the feces may affect the accuracy of the assay; results should always be interpreted in conjunction with clinical and epidemiologic findings.

Submission of more than one specimen for testing is not recommended.

Testing of colostomy, ileostomy, or colonoscopically collected specimens has not been validated.

Patients may asymptotically carry *Clostridioides difficile*; clinical correlation is needed when deciding how to manage patients with a positive test result.

Repeat testing **should not be performed** on specimens collected less than 7 days apart.

### Supportive Data

Results of the polymerase chain reaction (PCR) assay were compared with those of *Clostridioides difficile* toxin-detecting enzyme immunoassays (EIA) and culture of *C difficile*. Two hundred fecal specimens were studied in a blinded manner. *C difficile* was isolated from 49 specimens by culture and 44 of these were confirmed as containing 1 of the genes associated with toxin production (toxigenic culture). Using toxigenic culture as the "gold standard," the sensitivities and specificities, respectively, of the assays were 48% and 98% for the Premier Toxin A/B EIA (Meridian diagnostics); 48% and 99% for the ImmunoCard toxin A and B test (Meridian); 48% and 84% for the Xpect *C difficile* toxin A/B test (Remel); 32% and 100% for the Triage *C difficile* panel (for toxin A, Biosite Diagnostics); and 86% and 97% for the PCR assay. No cross-reactivity was observed in the PCR assay with a panel of 51 pathogens and normal flora, including other *C* species. The analytical sensitivity/limit of detection for the PCR assay was 35.8 cells/mcL in extracted fresh feces and 358 cells/mcL in extracted preserved feces.

### Clinical Reference

1. Aichinger E, Schleck CD, Harmsen WS, Nyre LM, Patel R: Nonutility of repeat laboratory testing for detection of *Clostridium difficile* by use of PCR or enzyme immunoassay. J Clin Microbiol. 2008;46:3795-3797
2. Verdoorn BP, Orenstein R, Rosenblatt JE, et al: High prevalence of *tcdC* deletion-carrying *Clostridium difficile* and lack of association with disease severity. Diagn Microbiol Infect Dis. 2010;66:24-28
3. Karre T, Sloan L, Patel R, Mandrekar J, Rosenblatt J: Comparison of two commercial molecular assays to a laboratory-developed molecular assay for diagnosis of *Clostridium difficile* infection. J Clin Microbiol. 2011;49:725-727
4. Lawson PA, Citron DM, Tyrrell KL, Finegold SM: Reclassification of *Clostridium difficile* as *Clostridioides difficile* (Hall and O'Toole 1935) Prevot 1938. Anaerobe. 2016 Aug;40:95-99. doi: 10.1016/j.anaerobe.2016.06.008
5. Oren A, Garrity GM: List of new names and new combinations previously effectively, but not validly, published. Int J Syst Evol Microbiol. 2016 Sep;66:3761-3764. doi: 10.1099/ijsem.0.001321

### Performance

#### Method Description

This method employs a target-specific detection system including polymerase chain reaction (PCR) primers, as well as fluorescent resonance energy transfer (FRET) hybridization probes targeting *tcdC*. The LightCycler instrument amplifies and monitors target nucleic acid sequences by fluorescence during PCR cycling. This is an automated PCR system that can rapidly detect amplified product development. The detection of amplified products is based on the FRET principle. For FRET product detection, a hybridization probe with a donor fluorophore, fluorescein, on the 3' end is excited by an external light source, which emits light that is absorbed by a second hybridization probe with an acceptor fluorophore, LC-Red 640, at the 5' end. The acceptor fluorophore then emits light of a different wavelength that is measured with a signal that is proportional to the amount of specific PCR product. The process is completed in a closed tube system. (Sloan LM, Duresko BJ, Gustafson DR, Rosenblatt JE: Comparison of real-time PCR for detection of the *tcdC* gene with four toxin immunoassays and culture in diagnosis of *Clostridium difficile* infection. J Clin Microbiol. 2008;46:1996-2001)

**PDF Report**

No

**Day(s) Performed**

Monday through Sunday

**Report Available**

1 to 2 days

**Specimen Retention Time**

7 days

**Performing Laboratory Location**

Rochester

**Fees and Codes****Fees**

- Authorized users can sign in to [Test Prices](#) for detailed fee information.
- Clients without access to Test Prices can contact [Customer Service](#) 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact [Customer Service](#).

**Test Classification**

This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

**CPT Code Information**

87493

**LOINC® Information**

Test ID	Test Order Name	Order LOINC Value
CDFRP	C. difficile Toxin PCR, F	54067-4

Result ID	Test Result Name	Result LOINC Value
SRC52	Specimen Source	31208-2
83124	Result	54067-4