Overview

Useful For
Rapid diagnosis of *Clostridium difficile*-associated diarrhea (CDAD) and pseudomembranous colitis (PMC)

Testing Algorithm
See Laboratory Testing for Infectious Causes of Diarrhea in Special Instructions.

Special Instructions
- [Laboratory Testing for Infectious Causes of Diarrhea](#)

Method Name
Real-Time Polymerase Chain Reaction (PCR) using LightCycler and Fluorescent Resonance Energy Transfer (FRET)

(PCR is utilized pursuant to a license agreement with Roche Molecular Systems, Inc.)

NY State Available
Yes

Specimen

Specimen Type
Fecal

Necessary Information
Specimen source is required.

Specimen Required
This test is validated for formed feces, although testing formed feces for *Clostridioides (Clostridium) difficile* is generally not clinically indicated.

The high sensitivity of amplification by PCR requires the specimen to be processed in an environment in which contamination of the specimen by *C 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
**Test Definition: CDFRP**

**C. difficile Toxin PCR, F**

**Collection Instructions:** Collect fresh fecal specimen and submit 1 gram or 5 mL in container with transport medium. Place feces in preservative within 2 hours of collection.

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

**Acceptable:**

**Specimen Type:** Unpreserved feces

**Supplies:**

Stool container, Small (Random), 4 oz (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 <7days

**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**

| Other | Feces in gel transport medium, ECOFIX preservative, formalin or PVA fixative |

**Specimen Stability Information**

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<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
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<tbody>
<tr>
<td>Fecal</td>
<td>Varies</td>
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**Clinical and Interpretive**

**Clinical Information**

*Clostridioides (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 been most frequently 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 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, and 4) toxin detection immunoassays, which are insensitive. The described PCR 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 PCR result for the presence of the gene regulating toxin production (*tcdC*) indicates the presence of *Clostridioides (Clostridium) difficile* and toxin A and/or B.

A negative result indicates the absence of detectable *C difficile tcdC* DNA in the specimen, but does not rule-out *C difficile* infection. False-negative results may occur due to inhibition of PCR, sequence variability underlying the primers or probes, or the presence of *C difficile* 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 1 specimen for testing is not recommended.

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

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

**Supportive Data**

Results of the PCR assay were compared with those of *Clostridioides (Clostridium) difficile* toxin-detecting EIAs 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/ml in extracted preserved feces.

**Clinical Reference**


**Performance**

**Method Description**

This method employs a target-specific detection system including 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, et al: 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) and Time(s) Test Performed**

Monday through Sunday

**Analytic Time**

Same day/1 day

**Maximum Laboratory Time**

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 U.S. Food and Drug Administration.

CPT Code Information
87493

LOINC® Information

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