

Certificate of Analysis for NR-49290

Clostridium difficile, Isolate 20120187

Catalog No. NR-49290

Product Description: Clostridium difficile (C. difficile; also referred to as Peptoclostridium difficile), isolate 20120187 was obtained from the stool of an elderly male patient with a healthcare-associated (HA) C. difficile infection in Tennessee, USA, in 2011. Isolate 20120187 was deposited as PCR ribotype 019, North American pulsed-field gel electrophoresis type 1 (NAP1), containing tcdA, tcdB and tcdC of the PaLoc operon, as well as the C. difficile binary toxin (CDT).

Lot¹: 63719805 Manufacturing Date: 28AUG2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ²	Report results	Irregular, flat, lobate, rough and gray (Figure 1)
Hemolysis ²	Report results	Non-hemolytic
Motility (wet mount)	Report results	Motile
Biochemical tests:	·	
Esculin hydrolysis ³	Positive	Positive
Gelatin hydrolysis ³	Positive	Positive
VITEK [®] MS (MALDI-TOF)	Consistent with C. difficile	Consistent with C. difficile
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1390 base pairs)	Consistent with C. difficile	Consistent with C. difficile
PCR Assay of Extracted DNA Presence of <i>C. difficile</i> -specific genes ⁴ Triose phosphate isomerase (<i>tpi</i>)	~ 230 base pair amplicon	~ 230 base pair amplicon
Presence of toxin genes ^{4,5}		
cdtB	~ 510 base pair amplicon	~ 510 base pair amplicon
tcdA (wild type)	~ 370 base pair amplicon	~ 370 base pair amplicon
tcdA (partial deletion)	No amplicon	~ 110 base pair amplicon ⁶
tcdB	~ 160 base pair amplicon	~ 160 base pair amplicon
Purity (post-freeze)		
Anaerobic growth ⁷	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Aerobic growth ⁸	No growth	No growth
Viability (post-freeze) ²	Growth	Growth

¹NR-49290 was produced by inoculation of the deposited material into Modified Reinforced Clostridial medium and incubated for 2 days at 37°C in an anaerobic atmosphere (< 0.5% O₂; Remel™ Anaero Pack-Anaero™ R681001). The material from the initial growth was passaged in Modified Reinforced Clostridial medium for 2 days at 37°C in an anaerobic atmosphere to produce this lot.

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²2 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood

³Tests were assessed after 7 days at 37°C in an anaerobic atmosphere. The gelatin tube was placed at 4°C for one hour prior to result determination.

⁴Lemee, L., et al. "Multiplex PCR Targeting *tpi* (Triose Phosphate Isomerase), *tcdA* (Toxin A), and *tcdB* (Toxin B) Genes for Toxigenic Culture of *Clostridium difficile*." <u>J. Clin. Microbiol.</u> 42 (2004): 5710-5714. PubMed: 15583303.

⁵Antikainen, J., et al. "Detection of Virulence Genes of *Clostridium difficile* by Multiplex PCR." <u>APMIS.</u> 117 (2009): 607-613. PubMed: 19664132.
⁶A faint band corresponding to the amplicon representing the partial deletion in *tcdA* was observed, even though this was not expected. This should be investigated further if the disposition of *tcdA* is important for your intended use.

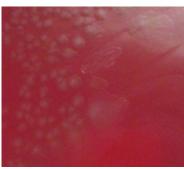
⁷Purity of this lot was assessed for 7 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.

⁸Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere with 5% CO₂ on Tryptic Soy agar with 5% defibrinated sheep blood.



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Figure 1: Colony Morphology



Date: 14 JAN 2016

Signature:

BEI Resources Authentication

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