Certificate of Analysis for NR-49277

Clostridioides difficile, Isolate 20100502

Catalog No. NR-49277

Product Description:

Clostridioides difficile (C. difficile), isolate 20100502 was isolated from the stool of an older adult male patient with a community-associated (CA) *C. difficile* infection in Colorado, USA, in 2010. Previously referred to as *Clostridium difficile*, the genus and species have been reclassified and the designation on the vial label refers to the old nomenclature. Isolate 20100502 was deposited as PCR ribotype 019, North American pulsed-field gel electrophoresis 1 (NAP1), containing *tcdA*, *tcdB* and *tcdC* of the PaLoc operon, as well as the *C. difficile* binary toxin (CDT). NR-49277 was produced by inoculation of BEI Resources seed lot 63719751 into Modified Reinforced Clostridial broth and incubated for 3 days at 37°C in an anaerobic atmosphere (< 5% O_2; Remel™ Pack-Anaero™). The material from the initial growth was passaged once in Modified Reinforced Clostridial broth for 3 days at 37°C in an anaerobic atmosphere to produce this lot.

Lot: 70063252 **Manufacturing Date: 11SEP2023**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology 3 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood	Report results	Irregular, flat, lobate and gray (Figure 1)
Motility (wet mount)	Report results	Motile
Hemolysis 3 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood	Report results	Non-hemolytic
VITEK® MS (MALDI-TOF)	*C. difficile*	*C. difficile* (99.9%)
Biochemical Characterization		
Esculin hydrolysis[1]	Positive	Positive
Gelatin hydrolysis[1]	Positive	Positive
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1440 base pairs)	≥ 99% sequence identity to *C. difficile*, strain 20100502 (GenBank: MTVQ01000002.1)	100% sequence identity to *C. difficile*, strain 20100502 (GenBank: MTVQ01000002.1)
PCR Assay of Extracted DNA		
Presence of *C. difficile*-specific genes[2]		
Triose phosphate isomerase (*tpi*)	~ 230 base pair amplicon	~ 230 base pair amplicon
Presence of toxin genes[2,3]		
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[4]
tcdB	~ 160 base pair amplicon	~ 160 base pair amplicon
Purity (post-freeze)		
Anaerobic 7 days at 37°C on Tryptic Soy agar with 5% defibrinated sheep blood	Report results	Growth consistent with expected colony morphology
Aerobic with 5% CO_2 7 days at 37°C on Tryptic Soy agar with 5% defibrinated sheep blood	Report results	No growth
Viability (post-freeze)		
3 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood	Growth	Growth

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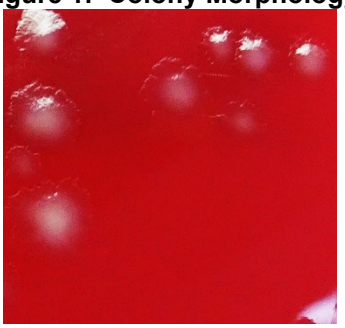
[1]Tests were assessed after 7 days at 37°C in an anaerobic atmosphere.
[2]Lemee, L., et al. "Multiplex PCR Targeting *tpi* (Triose Phosphate Isomerase), *tcdA* (Toxin A), and *tcdB* (Toxin B) Genes for Toxigenic Culture of *Clostridium difficile*." J. Clin. Microbiol. 42 (2004): 5710-5714. PubMed: 15583303.
[3]Antikainen, J., et al. "Detection of Virulence Genes of *Clostridium difficile* by Multiplex PCR." APMIS. 117 (2009): 607-613. PubMed: 19664132.
[4]A 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.

Figure 1: Colony Morphology



/Sonia Bjorum Brower/
Sonia Bjorum Brower 20 DEC 2023
Technical Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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