SUPPORTING INFECTIOUS DISEASE RESEARCH

Clostridium difficile, Isolate 20110992

Catalog No. NR-49319

Product Description: Clostridium difficile (C. difficile; also referred to as Peptoclostridium difficile), isolate 20110992 was obtained from the stool of an elderly male patient with a community-associated (CA) C. difficile infection in midwestern USA in 2011. Isolate 20110992 was deposited as PCR ribotype 106, North American pulsed-field gel electrophoresis type 11 (NAP11), containing *tcdA*, *tcdB* and *tcdC* of the PaLoc operon. This isolate is reported to be negative for the C. difficile binary toxin (CDT).

Lot¹: 63719903

Manufacturing Date: 01OCT2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ²	Report results	Irregular, flat, lobate and gray (Figure 1)
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	C. difficile (99.9%)
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene	≥ 99% sequence identity to	99.9% sequence identity to
(~ 1450 base pairs)	C. difficile type strain	C. difficile type strain
	(GenBank: CP011968.1)	(GenBank: CP011968.1)
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	No amplicon	No amplicon
<i>tcdA</i> (wild type)	~ 370 base pair amplicon	~ 370 base pair amplicon
tcdA (partial deletion)	No amplicon	No amplicon
tcdB	~ 160 base pair amplicon	~ 160 base pair amplicon
Purity (post-freeze)		
Anaerobic growth ⁶	Consistent with expected colony	Consistent with expected colony
v	morphology	morphology
Aerobic growth ⁷	No growth	No growth
Viability (post-freeze) ²	Growth	Growth

¹NR-49319 was produced by inoculation of the deposited material into Modified Reinforced Clostridial medium and incubated for 1 day at 37°C in an anaerobic atmosphere (< 0.5% O₂; Remel[™] Anaero Pack-Anaero[™]). The material from the initial growth was passaged once in Modified Reinforced Clostridial medium for 1 day at 37°C in an anaerobic atmosphere to produce this lot.

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*." J. Clin. Microbiol. 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. ⁶Purity of this lot was assessed for 2 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.

⁷Purity of this lot was assessed for 9 days at 37°C in an aerobic atmosphere with 5% CO₂ on Tryptic Soy agar with 5% defibrinated sheep blood.

biei resources

Certificate of Analysis for NR-49319

SUPPORTING INFECTIOUS DISEASE RESEARCH

Figure 1: Colony Morphology



Date: 14 OCT 2016

Signature: Jack Cuch

BEI Resources Authentication

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.

ATCC[®] is a trademark of the American Type Culture Collection. You are authorized to use this product for research use only. It is not intended for human use.

