

Certificate of Analysis for NR-49312

Clostridium difficile, Isolate 20110960

Catalog No. NR-49312

Product Description: Clostridium difficile (C. difficile), isolate 20110960 was obtained from the stool of an elderly male patient with a healthcare-associated (HA) C. difficile infection in Minnesota, USA, in 2011. Isolate 20110960 was deposited as PCR ribotype 017, North American pulsed-field gel electrophoresis type 9 (NAP9), containing tcdA, tcdB and tcdC of the PaLoc operon. This isolate is reported to be negative for the C. difficile binary toxin (CDT).

Lot¹: 63719960 Manufacturing Date: 02SEP2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ²	Report results	Irregular, raised, undulate, opaque 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 <i>C. difficile</i>
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene	Consistent with C. difficile	Consistent with C. difficile
(~ 1410 base pairs)		
PCR Assay of Extracted DNA		
Presence of <i>C. difficile</i> -specific genes ⁴		
Triose phosphate isomerase (tpi)	~ 230 base pair amplicon	~ 230 base pair amplicon
Presence of toxin genes ^{4,5}		
cdtB	No amplicon	No amplicon
tcdA (wild type)	No amplicon	No amplicon
tcdA (partial deletion) tcdB	~ 110 base pair amplicon	~ 110 base pair amplicon
	~ 160 base pair amplicon	~ 160 base pair amplicon
Purity (post-freeze)		
Anaerobic growth ⁶	Growth consistent with expected	Growth consistent with expected
A arabia arough ⁷	colony morphology	colony morphology
Aerobic growth ⁷	No growth	No growth
Viability (post-freeze) ²	Growth	Growth

¹NR-49312 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™ R681001). The material from the initial growth was passaged in Modified Reinforced Clostridial medium for 1 day at 37°C in an anaerobic atmosphere to produce this lot.

BEI Resources

www.beiresources.org

E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

²1 day 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.

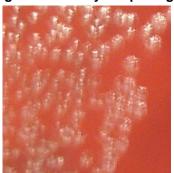
⁶Purity of this lot was assessed for 7 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.

⁷Purity 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.



Certificate of Analysis for NR-49312

Figure 1: Colony Morphology



Date: 01 DEC 2015

Signature:

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.

BEI Resources www.beiresources.org E-mail: contact@beiresources.org
Tel: 800-359-7370

Fax: 703-365-2898