b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49329

Clostridium difficile, Isolate 20121013

Catalog No. NR-49329

Product Description: *Clostridium difficile* (*C. difficile*; also referred to as *Peptoclostridium difficile*), isolate 20121013 was obtained from the stool of an elderly female patient with a healthcare-associated (HA) *C. difficile* infection in northeastern USA, in 2011. Isolate 20121013 was deposited as PCR ribotype 014, North American pulsed-field gel electrophoresis unnamed type C, containing *tcdA*, *tcdB* and *tcdC* of the PaLoc operon. This isolate is reported to be negative for the *C. difficile* binary toxin (CDT).

Lot[1]: 63719926 **Manufacturing Date: 18SEP2015**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology[2]	Report results	Irregular, flat, undulate, rough and gray (Figure 1)
Hemolysis[2]	Non-hemolytic	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*	*C. difficile* (99.9%)
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1390 base pairs)	≥ 99% sequence identity to *C. difficile* type strain	99% sequence identity to AB075770 (ATCC® 9689™)
PCR Assay of Extracted DNA		
Presence of *C. difficile*-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)	~ 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]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Aerobic growth[7]	No growth	No growth
Viability (post-freeze)[2]	Growth	Growth

[1]NR-49329 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% O_2; Remel™ Anaero Pack-Anaero™ R681001). The material from the initial growth was passaged in Tryptic Soy agar with 5% defibrinated sheep blood kolles for 2 days at 37°C in an anaerobic atmosphere to produce this lot.
[2]1 day at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood
[3]Tests 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.
[4]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.
[5]Antikainen, J., et al. "Detection of Virulence Genes of *Clostridium difficile* by Multiplex PCR." APMIS. 117 (2009): 607-613. PubMed: 19664132.
[6]Purity of this lot was assessed for 7 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.
[7]Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere with 5% CO_2 on Tryptic Soy agar with 5% defibrinated sheep blood.

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www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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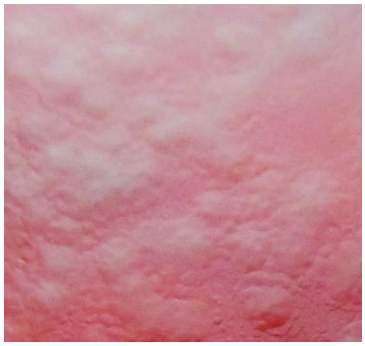
Figure 1: Colony Morphology



Date: 11 FEB 2016 **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.

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E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898