b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-43531

Peptoclostridium difficile, Strain CD149

Catalog No. NR-43531

Product Description: *Peptoclostridium difficile* (*P. difficile*; also referred to as *Clostridium difficile*), strain CD149 is a toxigenic strain isolated in March 2010 from the stool of a human patient diagnosed with an acute *Clostridium difficile* infection in Ann Arbor, Michigan, USA.

Lot[1]: 63261379 **Manufacturing Date: 21JAN2015**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology[2]	Report results	Irregular, flat, undulate, rugose and gray (Figure 1)
Hemolysis[2]	Report results	Non-hemolytic
Motility (wet mount)	Report results	Motile
Biochemical tests:		
Esculin hydrolysis	Positive	Positive
Gelatin hydrolysis	Positive	Positive
VITEK® MS (MALDI-TOF)	Consistent with *P. difficile*	Consistent with *P. difficile*
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 860 base pairs)	Consistent with *P. difficile*	Consistent with *P. difficile*[3]
PCR Assay of Extracted DNA		
Presence of *P. difficile*-specific genes[4]		
Triose phosphate isomerase (*tpi*)	~ 230 base pairs amplicon	~ 230 base pairs amplicon
Presence of toxin genes[4]		
Toxin A (*tcdA*)	~ 369 base pairs amplicon	~ 369 base pairs amplicon
Toxin B (*tcdB*)	~ 160 base pairs amplicon	~ 160 base pairs amplicon
Purity (post-freeze)		
Anaerobic growth[5]	Growth consistent with *P. difficile*	Growth consistent with *P. difficile*
Aerobic growth[6]	No growth	No growth
Viability (post-freeze)[2]	Growth	Growth

[1]NR-43531 was produced by inoculation of the deposited material into Modified Reinforced Clostridial medium and incubated for 22 hours at 37°C in an anaerobic atmosphere (< 0.5% O_2; Remel™ Anaero Pack-Anaero™ R681001). The material from the initial growth was passaged once in Modified Reinforced Clostridial medium for 23 hours under propagation conditions to produce this lot.

[2]24 hours on Tryptic Soy agar with 5% defibrinated sheep blood under propagation conditions

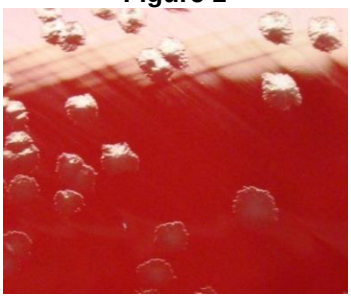
[3]≥ 99.7% identical to GenBank: AVHU010000071.1 (*P. difficile*, strain CD149)

[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]Purity of this lot was assessed for 7 days on Tryptic Soy agar with 5% defibrinated sheep blood under propagation conditions.

[6]Purity of this lot was assessed for 7 days on Tryptic Soy agar with 5% defibrinated sheep blood in an aerobic atmosphere with 5% CO_2.

Figure 1



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

Certificate of Analysis for NR-43531

Date: 17 APR 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.

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