SUPPORTING INFECTIOUS DISEASE RESEARCH

Peptoclostridium difficile, Strain DA00065

Catalog No. NR-43567

Product Description: *Peptoclostridium difficile* (*P. difficile*; also referred to as *Clostridium difficile*), strain DA00065 is a toxigenic strain isolated in October 2010 from the stool of a human patient diagnosed with an acute *Clostridium difficile* infection in Ann Arbor, Michigan, USA.

Lot¹: 63312209

Manufacturing Date: 20FEB2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology ²	Report results	Irregular, flat, undulate, smooth 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 (~ 1450 base pairs)	Consistent with <i>P. difficile</i>	Consistent with <i>P. difficile</i> ³
PCR Assay of Extracted DNA		
Presence of <i>P. difficile</i> -specific genes ⁴		
Triose phosphate isomerase (<i>tpi</i>)	~ 230 base pairs amplicon	~ 230 base pairs amplicon
Presence of toxin genes ⁴		
Toxin A (<i>tcdA</i>)	~ 110 base pairs amplicon	~ 110 base pairs amplicon ^{4,5}
Toxin B (<i>tcdB</i>)	~ 160 base pairs amplicon	~ 160 base pairs amplicon
Purity (post-freeze)		
Anaerobic growth ⁶	Growth consistent with P. difficile	Growth consistent with <i>P. difficile</i>
Aerobic growth ⁷	No growth	No growth
Viability (post-freeze) ²	Growth	Growth

NR-43567 was produced by inoculation of the deposited material into Modified Reinforced Clostridial medium and incubated for 47 hours at 37°C in an anaerobic atmosphere (< 0.5% O₂; Remel[™] Anaero Pack-Anaero[™] R681001). The material from the initial growth was passaged once in Modified Reinforced Clostridial medium for 24 hours under propagation conditions to produce this lot.

²43 hours on Tryptic Soy agar with 5% defibrinated sheep blood under propagation conditions

³≥ 99.7% identical to GenBank: AVIU010000038.1 (*P. difficile*, strain DA00065)

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.

⁵The observed ~ 110-bp amplicon is consistent with a partial deletion of the *tcdA* gene.

⁶Purity of this lot was assessed for 7 days on Tryptic Soy agar with 5% defibrinated sheep blood under propagation conditions.

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

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Certificate of Analysis for NR-43567

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Date: 18 MAY 2015

Signature:

BEI Resources Authentication

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