b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49313

Clostridium difficile, Isolate 20110963

Catalog No. NR-49313

Product Description: *Clostridium difficile* (*C. difficile*), isolate 20110963 was obtained from the stool of an elderly female patient with a healthcare-associated (HA) *C. difficile* infection in Minnesota, USA, in 2011. Isolate 20110963 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).

Lot[1]: 63719864 **Manufacturing Date: 17SEP2015**

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 *C. difficile*
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1420 base pairs)	Consistent with *C. difficile*	Consistent with *C. difficile*
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)	No amplicon	No amplicon
tcdA (partial deletion)	~ 110 base pair amplicon	~ 110 base pair 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-49313 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 Modified Reinforced Clostridial medium for 1 day 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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Figure 1: Colony Morphology

Date: 02 DEC 2015 **Signature:**

BEI Resources Authentication

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