

## Certificate of Analysis for NR-49314

## Clostridium difficile, Isolate 20121412

## Catalog No. NR-49314

**Product Description:** Clostridium difficile (C. difficile), isolate 20121412 was obtained from the stool of a young female patient with a community-associated (CA) C. difficile infection in Gerogia, USA, in 2011. Isolate 20121412 was deposited as PCR ribotype 047, 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<sup>1</sup>: 63719888 Manufacturing Date: 16SEP2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology <sup>2</sup>	Report results	Irregular, raised, undulate, opaque and gray (Figure 1)
Hemolysis <sup>2</sup>	Report results	Non-hemolytic
Motility (wet mount)	Report results	Motile
Biochemical tests:		
Esculin hydrolysis <sup>3</sup>	Positive	Positive
Gelatin hydrolysis <sup>3</sup>	Positive	Positive
VITEK <sup>®</sup> MS (MAĹDI-TOF)	Consistent with C. difficile	Consistent with C. difficile
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1340 base pairs)	Consistent with C. difficile	Consistent with C. difficile
PCR Assay of Extracted DNA  Presence of <i>C. difficile</i> -specific genes <sup>4</sup> Triose phosphate isomerase ( <i>tpi</i> )  Presence of toxin genes <sup>4,5</sup>	~ 230 base pair amplicon	~ 230 base pair amplicon
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 <sup>6</sup>	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Aerobic growth <sup>7</sup>	No growth	No growth
Viability (post-freeze) <sup>2</sup>	Growth	Growth

<sup>&</sup>lt;sup>1</sup>NR-49314 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.

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<sup>&</sup>lt;sup>2</sup>1 day at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood

<sup>&</sup>lt;sup>3</sup>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.

<sup>&</sup>lt;sup>4</sup>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.

<sup>&</sup>lt;sup>5</sup>Antikainen, J., et al. "Detection of Virulence Genes of Clostridium difficile by Multiplex PCR." APMIS. 117 (2009): 607-613. PubMed: 19664132.

<sup>&</sup>lt;sup>6</sup>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.

<sup>&</sup>lt;sup>7</sup>Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> on Tryptic Soy agar with 5% defibrinated sheep blood.



## **Certificate of Analysis for NR-49314**

Figure 1: Colony Morphology



**Date:** 13 JAN 2016

Signature:

**BEI Resources Authentication** 

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