

## Certificate of Analysis for NR-13427

## Clostridium difficile, Isolate 1

## Catalog No. NR-13427

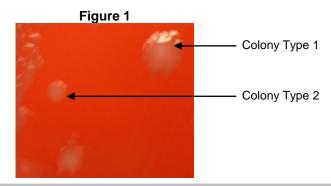
**Product Description:** Clostridium difficile (C. difficile), isolate 1 was obtained from a human patient from the Mid-Atlantic region of the United States in 2008/2009.

Lot<sup>1</sup>: 58807148 Manufacturing Date: 14JAN2010

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis <sup>2</sup>		
Cellular morphology	Gram-positive rod	Gram-positive rod
Colony morphology <sup>3,4</sup>	Report results	Colony type 1: Irregular, flat, erose, opaque, glossy and gray (Figure 1) Colony type 2: Irregular, flat, erose, opaque, glossy and white (Figure 1)
Hemolysis <sup>4</sup>	Report results	Non-hemolytic
Obligate anaerobe	Positive	Positive
Chartreuse fluorescence on anaerobic blood agar <sup>5</sup>	Positive	Positive
Biochemical tests:		
RapID™ ANA II Panel	Consistent with C. difficile	Consistent with C. difficile
Esculin hydrolysis	Positive	Positive
Gelatin hydrolysis	Positive	Positive
Catalase activity	Negative	Negative
Lecithinase	Negative	Negative
Lipase	Negative	Negative
Nitrate reduction	Negative	Negative
Hydrogen sulfide production	Negative	Negative
Genotypic Analysis		
Sequencing of 16S Ribosomal RNA Gene	Consistent with C. difficile	Consistent with C. difficile
(~ 1370 base pairs)		
PCR Assay of Extracted DNA		
16S ribosomal RNA gene	~ 1500 bp amplicon	~ 1500 bp amplicon
Viability (post-freeze) <sup>4</sup>	Growth	Growth

The deposited material was inoculated into Modified Reinforced Clostridial Broth (ATCC medium 2107) and incubated for 48 hours at 37°C and anaerobic atmosphere (80% N<sub>2</sub>:10% CO<sub>2</sub>:10% H<sub>2</sub>). The material from the initial growth was passaged three times in Modified Reinforced Clostridial Broth at 37°C and anaerobic atmosphere [80% N<sub>2</sub>:10% CO<sub>2</sub>:10% H<sub>2</sub>]. NR-13427 was produced from the third passage.

<sup>&</sup>lt;sup>5</sup>48 hours at 37°C and anaerobic atmosphere (80% N<sub>2</sub>:10% CO<sub>2</sub>:10% H<sub>2</sub>) on CDC anaerobic blood agar



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<sup>&</sup>lt;sup>2</sup>Specifications described in Holdeman, L. V., E. P. Cato, and W. E. C. Moore, Eds. <u>Anaerobe Laboratory Manual</u>. 4th ed., Blacksburg: Virginia Polytechnic Institute and State University, 1977.

<sup>&</sup>lt;sup>3</sup>Two colony types were observed. Plating of the individual colony types showed that they reverted to the mixed colony type. The 16S gene of each colony type was sequenced and determined to be 100% identical.

<sup>&</sup>lt;sup>4</sup>48 hours at 37°C and anaerobic atmosphere (80% N₂:10% CO₂:10% H₂) on Tryptic Soy Agar with 5% defibrinated sheep blood



## Certificate of Analysis for NR-13427

**Date:** 27 JUL 2010 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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