

Certificate of Analysis for NR-13437

Clostridium difficile, Isolate 11

Catalog No. NR-13437

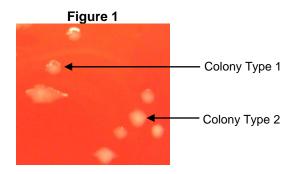
Product Description: Clostridium difficile (C. difficile), isolate 11 was obtained from a human patient from the Mid-Atlantic region of the United States in 2008/2009.

Lot¹: 59147480 Manufacturing Date: 14APR2010

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ²		
Cellular morphology	Gram-positive rod	Gram-positive rod
Colony morphology ^{3,4}	Report results	Colony type 1: Irregular, flat, erose, opaque and gray (Figure 1) Colony type 2: Irregular, flat, erose, opaque and white (Figure 1)
Obligate Anaerobe	Positive	Positive
Hemolysis ⁴	Report results	Non-hemolytic
Chartreuse fluorescence on anaerobic blood agar ⁴	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
Genotypic Analysis Sequencing of 16S Ribosomal RNA Gene (~ 1410 base pairs)	Consistent with C. difficile	Consistent with C. difficile
PCR Assay of Extracted DNA 16S ribosomal RNA gene	~ 1500 bp amplicon	~ 1500 bp amplicon
Viability (post-freeze) ⁴	Growth	Growth

¹The deposited material was inoculated into Modified Reinforced Clostridial Broth (<u>ATCC medium 2107</u>) and incubated for 48 hours at 37°C and anaerobic atmosphere (80% N₂:10% CO₂:10% H₂). The material from the initial growth was passaged three times in Modified Reinforced Clostridial Broth at 37°C and anaerobic atmosphere [80% N₂:10% CO₂:10% H₂]. NR-13437 was produced from the fourth passage.

⁴⁴⁸ hours at 37°C and anaerobic atmosphere (80% N₂:10% CO₂:10% H₂) on CDC anaerobic blood agar



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²Specifications 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.

³Two colony types were observed. The 16S ribosomal RNA gene of each colony type was sequenced and found to be consistent with C. difficile.



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Date: 21 OCT 2010 Signature:

Title: Technical Manager, BEI Authentication or designee

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