

Certificate of Analysis for NR-13436

Clostridium difficile, Isolate 10

Catalog No. NR-13436

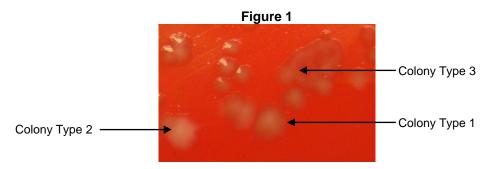
Product Description: Clostridium difficile (C. difficile), isolate 10 was obtained from a human patient from the Mid-Atlantic region of the United States in 2008/2009.

Lot¹: 59147481 Manufacturing Date: 03JUN2010

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, undulate, rough, opaque and gray (Figure 1)
		Colony type 2: Irregular, flat, undulate, rough, opaque and white (Figure 1)
		Colony type 3: Irregular, flat, undulate, mucoid, opaque and gray (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
Genotypic Analysis		
Sequencing of 16S Ribosomal RNA Gene (~ 1350 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-13436 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.

³Three 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.



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Date: 21 OCT 2010

Signature:

Title:

Technical Manager, BEI Authentication or designee

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