

Certificate of Analysis for NR-13553

Clostridium difficile, Isolate 13

Catalog No. NR-13553

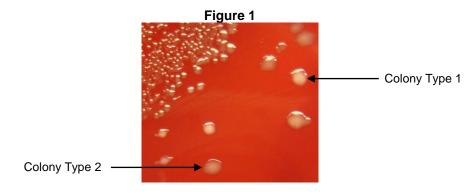
Product Description: Clostridium difficile (C. difficile), isolate 13 was obtained from a human patient from the Mid-Atlantic region of the United States in 2008/2009.

Lot¹: 59147477 Manufacturing Date: 14APR2010

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ²		
Cellular morphology	Gram-positive rod	Gram-positive rod
Colony morphologies ^{3,4}	Report results	Colony type 1: Irregular, flat, erose, undulate, opaque, glistening and gray (Figure 1)
		Colony type 2: Irregular, flat, erose, undulate, opaque, glistening and white (Figure 1)
Obligate Anaerobe	Positive	Positive
Hemolysis ⁴	Report results	Non-hemolytic
Chartreuse fluorescence on anaerobic blood agar ⁴ Biochemical tests:	Positive	Positive
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 (~ 1370 base pairs)	Consistent with C. difficile	Consistent with C. difficile
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-13553 was produced from the fourth passage.

⁴⁸ 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. 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 found to be consistent with *C. difficile*.



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Date: 10 MAY 2011 Signature:

Title: Technical Manager, BEI Authentication or designee

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