

Certificate of Analysis for NR-13435

Clostridioides difficile, Isolate 9

Catalog No. NR-13435

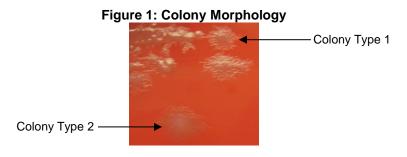
Product Description: Clostridioides difficile (C. difficile), isolate 9 was obtained from a human patient from the Mid-Atlantic region of the United States in 2008/2009. Previously referred to as Clostridium difficile, this genus has been reclassified and the genus designation on the vial label refers to the old nomenclature.

Lot¹: 59147482 Manufacturing Date: 05MAY2010

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, undulate, opaque, glistening and gray (Figure 1)
		Colony type 2: Irregular, flat, undulate, opaque, glistening and white (Figure 1)
Obligate Anaerobe	Positive	Positive
Hemolysis ⁴	Report results	Non-hemolytic
Chartreuse fluorescence on Anaerobic Blood agar4	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 (~ 1430 base pairs)	Consistent with C. difficile	Consistent with C. difficile
Viability (post-freeze) ⁴	Growth	Growth

¹ The deposited material was inoculated into Modified Reinforced Clostridial broth and incubated for 2 days at 37°C in an 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 in an anaerobic atmosphere. NR-13435 was produced from the fourth passage.

⁴2 days at 37°C in an anaerobic atmosphere on CDC Anaerobic Blood agar



/Heather Couch/ **Heather Couch**

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Program Manager or designee, ATCC Federal Solutions

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E-mail: contact@beiresources.org www.beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

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.