

Certificate of Analysis for NR-13430

Clostridium difficile, Isolate 4

Catalog No. NR-13430

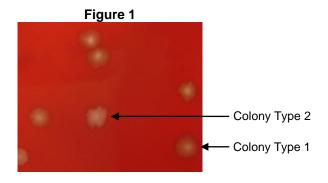
Product Description: Clostridium difficile (C. difficile), isolate 4 was obtained from a human patient from the Mid-Atlantic region of the United States in 2008/2009.

Lot¹: 59062322 Manufacturing Date: 31MAR2010

| 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 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 (~ 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 |

¹NR-13430 was produced by inoculation of the deposited material 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-13430 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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