

***Clostridium difficile*, Isolate 20110241**

Catalog No. NR-49299

Product Description: *Clostridium difficile* (*C. difficile*; also referred to as *Peptoclostridium difficile*), isolate 20110241 was obtained from the stool of an older female patient with a community-associated (CA) *C. difficile* infection in midwestern USA in 2010. Isolate 20110241 was deposited as PCR ribotype 020, North American pulsed-field gel electrophoresis type 4 (NAP4), containing *tcdA* and *tcdB* of the PaLoc operon. This isolate is reported to be negative for the *C. difficile* binary toxin (CDT).

Lot¹: 63950677

Manufacturing Date: 08APR2016

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Cellular morphology Colony morphology ² Hemolysis ² Motility (wet mount) Biochemical tests: Esculin hydrolysis ³ Gelatin hydrolysis ⁴ VITEK [®] MS (MALDI-TOF)	Gram-positive rods Report results Report results Report results Positive Positive <i>C. difficile</i>	Gram-positive rods Irregular, flat, lobate, translucent and gray (Figure 1) Non-hemolytic Motile Positive Positive <i>C. difficile</i> (99.9%) ⁵
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1400 base pairs)	≥ 99% sequence identity to <i>C. difficile</i> type strain (GenBank: CP011968.1)	99.9% sequence identity to <i>C. difficile</i> type strain (GenBank: CP011968.1)
PCR Assay of Extracted DNA Presence of <i>C. difficile</i> -specific genes ⁶ Triose phosphate isomerase (<i>tpi</i>) Presence of toxin genes ^{6,7} <i>cdtB</i> <i>tcdA</i> (wild type) <i>tcdA</i> (partial deletion) <i>tcdB</i>	~ 230 base pair amplicon ~ 510 base pair amplicon ~ 370 base pair amplicon No amplicon ~ 160 base pair amplicon	~ 230 base pair amplicon No amplicon ~ 370 base pair amplicon No amplicon ~ 160 base pair amplicon
Purity (post-freeze) Anaerobic growth ⁸ Aerobic growth ⁹	Growth consistent with expected morphology No growth	Growth consistent with expected morphology No growth
Viability (post-freeze)²	Growth	Growth

¹The deposited material was inoculated into Modified Reinforced Clostridial medium, which was passaged four times at 37°C in an anaerobic atmosphere (< 0.5% O₂; Remel™ Anaero Pack-Anaero™ R681001) and preserved in 10% glycerol. NR-49299 was produced by inoculation of the preserved material into Modified Reinforced Clostridial medium and incubated for 2 days at 37°C in an anaerobic atmosphere. The material from the initial growth was passaged once in Modified Reinforced Clostridial medium for 1 day at 37°C in an anaerobic atmosphere to produce this lot.

²3 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood

³Esculin hydrolysis was assessed after 7 days at 37°C in an anaerobic atmosphere.

⁴Gelatin hydrolysis was assessed after 2 days at 37°C in an anaerobic atmosphere. The gelatin tube was placed at 4°C for one hour prior to result determination.

⁵Percent probabilities above 90% indicate a close match to the typical biochemical pattern for the given organism, with a percent probability of 99% being a perfect match between the test reaction pattern and the unique biochemical pattern of the given organism or organism group. For additional information, please refer to O'Hara, C.M. and J. M. Miller. "Evaluation of the VITEK 2 ID-GNB Assay for Identification of Members of the Family Enterobacteriaceae and Other Nonenteric Gram-Negative Bacilli and Comparison with the VITEK GNI+ Card." *J. Clin. Microbiol.* 41 (2003): 2096-2101. PubMed: 12734254.

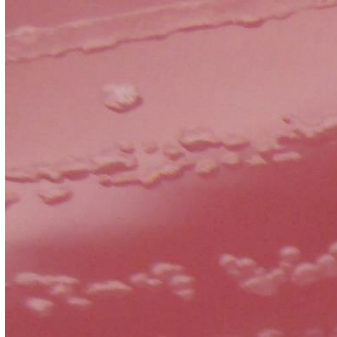
⁶Lemee, L., et al. "Multiplex PCR Targeting *tpi* (Triose Phosphate Isomerase), *tcdA* (Toxin A), and *tcdB* (Toxin B) Genes for Toxigenic Culture of *Clostridium difficile*." *J. Clin. Microbiol.* 42 (2004): 5710-5714. PubMed: 15583303.

⁷Antikainen, J., et al. "Detection of Virulence Genes of *Clostridium difficile* by Multiplex PCR." *APMIS*. 117 (2009): 607-613. PubMed: 19664132.

⁸Purity of this lot was assessed for 3 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.

⁹Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere with 5% CO₂ on Tryptic Soy agar with 5% defibrinated sheep blood.

Figure 1: Colony Morphology



Date: 03 NOV 2016

Signature:

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