

***Clostridium difficile*, Isolate 20120020**

Catalog No. NR-49307

Product Description: *Clostridium difficile* (*C. difficile*; also referred to as *Peptoclostridium difficile*), isolate 20120020 was obtained from the stool of an elderly female patient with a community-associated (CA) *C. difficile* infection in northeastern USA, in 2011. Isolate 20120020 was deposited as PCR ribotype 002, North American pulsed-field gel electrophoresis type 6 (NAP6), containing *tcdA*, *tcdB* and *tcdC* of the PaLoc operon. This isolate is reported to be negative for the *C. difficile* binary toxin (CDT).

Lot¹: 63719847

Manufacturing Date: 27AUG2015

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis Cellular morphology Colony morphology ^{2,3} Hemolysis ² Motility (wet mount) Biochemical tests: Esculin hydrolysis ³ Gelatin hydrolysis ³ VITEK [®] MS (MALDI-TOF)	Gram-positive rods Report results Non-hemolytic Report results Positive Positive Consistent with <i>C. difficile</i>	Gram-positive rods Colony type 1: Irregular, slight peaked, undulate, opaque and gray (Figure 1) Colony type 2: Circular, convex, entire, opaque and gray (Figure 2) Non-hemolytic Motile Positive Positive Consistent with <i>C. difficile</i> (99.9%)
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1410 base pairs)	≥ 99% sequence identity to <i>C. difficile</i> type strain	99% sequence identity to AB075770 (ATCC [®] 9689 [™])
PCR Assay of Extracted DNA Presence of <i>C. difficile</i> -specific genes ⁴ Triose phosphate isomerase (<i>tpi</i>) Presence of toxin genes ^{4,5} <i>cdtB</i> <i>tcdA</i> (wild type) <i>tcdA</i> (partial deletion) <i>tcdB</i>	~ 230 base pair amplicon No 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 ⁶ Aerobic growth ⁷	Growth consistent with expected colony morphology No growth	Growth consistent with expected colony morphology No growth
Viability (post-freeze)²	Growth	Growth

¹NR-49307 was produced by inoculation of the deposited material into Modified Reinforced Clostridial medium and incubated for 2 days at 37°C in an anaerobic atmosphere (< 0.5% O₂; Remel[™] Anaero Pack-Anaero[™] R681001). The material from the initial growth was passaged in Modified Reinforced Clostridial medium for 1 day at 37°C in an anaerobic atmosphere to produce this lot.

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

³Tests were assessed after 7 days at 37°C in an anaerobic atmosphere. The gelatin tube was placed at 4°C for one hour prior to result determination.

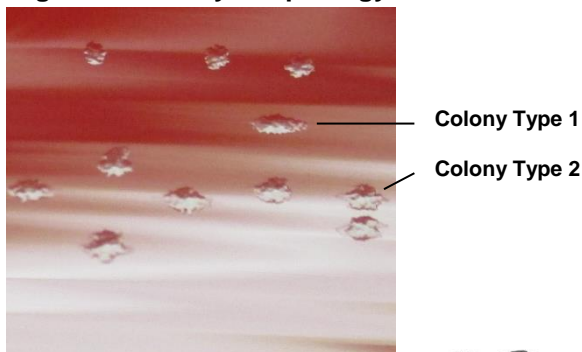
⁴Lemee, L., et al. "Multiplex PCR Targeting *tpi* (Triose Phosphate Isomerase), *tcdA* (Toxin A), and *tcdB* (Toxin B) Genes for Toxigenic Culture of *Clostridium difficile*." *J. Clin. Microbiol.* 42 (2004): 5710-5714. PubMed: 15583303.

⁵Antikainen, J., et al. "Detection of Virulence Genes of *Clostridium difficile* by Multiplex PCR." *APMIS*. 117 (2009): 607-613. PubMed: 19664132.

⁶Purity of this lot was assessed for 7 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.

⁷Purity 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: 22 FEB 2016

Signature:

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