

***Clostridium difficile*, Isolate 20110566**

Catalog No. NR-49300

Product Description: *Clostridium difficile* (*C. difficile*; also referred to as *Peptoclostridium difficile*), isolate 20110566 was obtained from the stool of a male patient with a community-associated (CA) *C. difficile* infection in northeastern USA in 2010. Isolate 20110566 was deposited as PCR ribotype 020, North American pulsed-field gel electrophoresis type 4 (NAP4), containing *tcdA*, *tcdB* and *tcdC* of the PaLoc operon. This isolate is reported to be negative for the *C. difficile* binary toxin (CDT).

Lot¹: 63950673

Manufacturing Date: 08JAN2016

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| Phenotypic Analysis Cellular morphology Colony morphology ² Hemolysis ² Motility (wet mount) Biochemical tests: Esculin hydrolysis ³ Gelatin hydrolysis ³ VITEK [®] MS (MALDI-TOF) | Gram-positive rods Report results Report results Report results Positive Positive Consistent with <i>C. difficile</i> | Gram-positive rods Irregular, flat, lobate, opaque and gray (Figure 1) Non-hemolytic Motile Positive Positive <i>C. difficile</i> (99.9%) |
| Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 910 base pairs) | ≥ 99% sequence identity to <i>C. difficile</i> type strain (GenBank: CP011968.1) | 100% 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 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 ~ 110 base pair amplicon ⁶ ~ 160 base pair amplicon |
| Purity (post-freeze) Anaerobic growth ⁷ Aerobic growth ⁸ | Growth consistent with expected colony morphology No growth | Growth consistent with expected colony morphology No growth |
| Viability (post-freeze)² | Growth | Growth |

¹*C. difficile*, isolate 20110566 was inoculated into Modified Reinforced Clostridial medium and grown for 2 days at 37°C in an anaerobic atmosphere (< 5% O₂; Remel™ Pack-Anaero™). The growth material was passaged once in Modified Reinforced Clostridial medium for 1 day at 37°C in an anaerobic atmosphere and preserved in 10% glycerol. Due to poor growth observed during quality control testing, the preserved material was inoculated in Modified Reinforced Clostridial medium. Medium inoculum was added to Tryptic Soy agar with 5% defibrinated sheep blood plates, which were grown for 7 days at 37°C in an anaerobic atmosphere. The growth material was passaged once in Modified Reinforced Clostridial medium for 1 day at 37°C in an anaerobic atmosphere and preserved in 10% glycerol. NR-49300 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 growth material was passaged once 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.

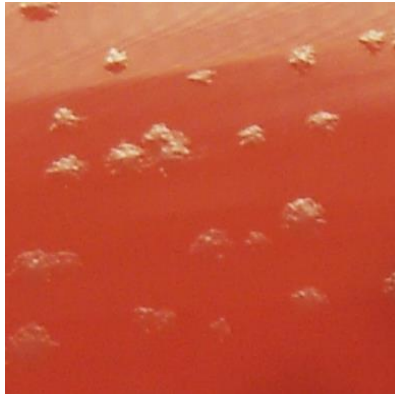
⁵Antikainen, J., et al. "Detection of Virulence Genes of *Clostridium difficile* by Multiplex PCR." *APMIS*. 117 (2009): 607-613. PubMed: 19664132.

⁶A faint band corresponding to the amplicon representing the partial deletion in *tcdA* was observed, even though this was not expected. This should be investigated further if the disposition of *tcdA* is important for your intended use.

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

⁸Purity 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: 24 JUN 2016

Signature:

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