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SUPPORTING INFECTIOUS DISEASE RESEARCH

# Certificate of Analysis for NR-49283

## *Clostridium difficile*, Isolate 20120013

## Catalog No. NR-49283

**Product Description:** *Clostridium difficile* (*C. difficile*; also referred to as *Peptoclostridium difficile*), isolate 20120013 was obtained from the stool of a young male patient with a community-associated (CA) *C. difficile* infection in northeastern USA, in 2011. Isolate 20120013 was deposited as PCR ribotype 027, North American pulsed-field gel electrophoresis type 1 (NAP1), containing *tcdA*, *tcdB* and *tcdC* (with 18 base pair deletion) of the PaLoc operon as well as the *C. difficile* binary toxin (CDT).

**Lot[1]: 63719786** **Manufacturing Date: 10SEP2015**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Phenotypic Analysis** | | |
| Cellular morphology | Gram-positive rods | Gram-positive rods |
| Colony morphology[2] | Report results | Irregular, slight peaked, undulate, opaque, rough and gray (Figure 1) |
| Hemolysis[2] | Non-hemolytic | Non-hemolytic |
| Motility (wet mount) | Report results | Motile |
| Biochemical tests: | | |
| Esculin hydrolysis[3] | Positive | Positive |
| Gelatin hydrolysis[3] | Positive | Positive |
| VITEK® MS (MALDI-TOF) | Consistent with *C. difficile* | *C. difficile* (99.9%) |
| **Genotypic Analysis** | | |
| Sequencing of 16S ribosomal RNA gene (~ 1390 base pairs) | > 99% sequence identity to *C. difficile* type strain | 99.9% sequence identity to CP011968.1 (ATCC® 9689™) |
| **PCR Assay of Extracted DNA** | | |
| Presence of *C. difficile*-specific genes[4] | | |
| Triose phosphate isomerase (*tpi*) | ~ 230 base pair amplicon | ~ 230 base pair amplicon |
| Presence of toxin genes[4,5] | | |
| *cdtB* | ~ 510 base pair amplicon | ~ 510 base pair amplicon |
| *tcdA* (wild type) | ~ 370 base pair amplicon | ~ 370 base pair amplicon |
| *tcdA* (partial deletion) | No amplicon | No amplicon |
| *tcdB* | ~ 160 base pair amplicon | ~ 160 base pair amplicon |
| **Purity (post-freeze)** | | |
| Anaerobic growth[6] | Growth consistent with expected morphology | Growth consistent with expected morphology |
| Aerobic growth[7] | No growth | No growth |
| **Viability (post-freeze)[2]** | Growth | Growth |

[1]NR-49283 was produced by inoculation of the deposited material into Modified Reinforced Clostridial medium and incubated for 1 day at 37°C in an anaerobic atmosphere (< 0.5% $O_2$; 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.
[2]1 day at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood
[3]Tests 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.
[4]Lemee, 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.
[5]Antikainen, J., et al. "Detection of Virulence Genes of *Clostridium difficile* by Multiplex PCR." APMIS. 117 (2009): 607-613. PubMed: 19664132.
[6]Purity of this lot was assessed for 10 days at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.
[7]Purity of this lot was assessed for 10 days at 37°C in an aerobic atmosphere with 5% $CO_2$ on Tryptic Soy agar with 5% defibrinated sheep blood.

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**Figure 1: Colony Morphology**

**Date:** 28 JAN 2016 **Signature:**

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.



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