

***Clostridium difficile*, Isolate 20120016**

**Catalog No. NR-49282**

**Product Description:** *Clostridium difficile* (*C. difficile*; also referred to as *Peptoclostridium difficile*), isolate 20120016 was obtained from the stool of a pediatric female patient with a community-associated (CA) *C. difficile* infection in New York, USA, in 2011. Isolate 20120016 was deposited as PCR ribotype 019, North American pulsed-field gel electrophoresis type 1 (NAP1), containing *tcdA*, *tcdB* and *tcdC* of the PaLoc operon, as well as the *C. difficile* binary toxin (CDT).

**Lot<sup>1</sup>: 63719785**

**Manufacturing Date: 02SEP2015**

TEST	SPECIFICATIONS	RESULTS
<b>Phenotypic Analysis</b> Cellular morphology Colony morphology <sup>2</sup>  Hemolysis <sup>2</sup> Motility (wet mount) Biochemical tests: Esculin hydrolysis <sup>3</sup> Gelatin hydrolysis <sup>3</sup> VITEK <sup>®</sup> MS (MALDI-TOF)	Gram-positive rods Report results  Report results Report results  Positive Positive Consistent with <i>C. difficile</i>	Gram-positive rods Irregular, slight peaked, undulate, rough, opaque and gray (Figure 1) Non-hemolytic Motile  Positive Positive Consistent with <i>C. difficile</i>
<b>Genotypic Analysis</b> Sequencing of 16S ribosomal RNA gene (~ 1390 base pairs)	Consistent with <i>C. difficile</i>	Consistent with <i>C. difficile</i>
<b>PCR Assay of Extracted DNA</b> Presence of <i>C. difficile</i> -specific genes <sup>4</sup> Triose phosphate isomerase ( <i>tpi</i> ) Presence of toxin genes <sup>4,5</sup> <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  ~ 510 base pair amplicon ~ 370 base pair amplicon ~ 110 base pair amplicon <sup>6</sup> ~ 160 base pair amplicon
<b>Purity (post-freeze)</b> Anaerobic growth <sup>7</sup>  Aerobic growth <sup>8</sup>	Growth consistent with expected colony morphology No growth	Growth consistent with expected colony morphology No growth
<b>Viability (post-freeze)<sup>2</sup></b>	Growth	Growth

<sup>1</sup>NR-49282 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<sub>2</sub>; 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.

<sup>2</sup>1 day at 37°C in an anaerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood

<sup>3</sup>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.

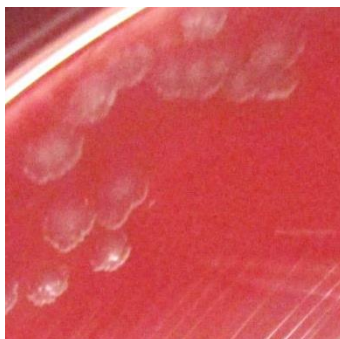
<sup>4</sup>Lemee, L., et al. "Multiplex PCR Targeting *tpi* (Triose Phosphate Isomerase), *tcdA* (Toxin A), and *tcdB* (Toxin B) Genes for Toxigenic Culture of *Clostridium difficile*." *J. Clin. Microbiol.* 42 (2004): 5710-5714. PubMed: 15583303.

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

<sup>6</sup>A 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.

<sup>7</sup>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.

<sup>8</sup>Purity of this lot was assessed for 10 days at 37°C in an aerobic atmosphere with 5% CO<sub>2</sub> on Tryptic Soy agar with 5% defibrinated sheep blood.

**Figure 1: Colony Morphology****Date:** 02 DEC 2015**Signature:**

A handwritten signature in black ink, which appears to read "David C. Cook".

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