Certificate of Analysis for NR-49066

Mycobacterium canettii, Strain 563

Catalog No. NR-49066

Product Description: *Mycobacterium canettii* (*M. canettii*), strain 563 is of unknown origin.

Lot[1]: 63453263 **Manufacturing Date: 13NOV2015**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2]		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology[3]	Report results	Irregular, slight peaked, undulate, rough and cream[4] (Figure 1)
Growth rate	≥ 7 days	21 days
Growth at 26°C	Negative	Negative
Growth at 37°C	Positive	Positive
Acid-fast stain	Positive (red colonies)	Positive (red colonies)
Pigmentation in the dark (Scotochromogen)	Negative (no pigment)	Negative (no pigment)
Photoinduction for 1 hour (Photochromogen)	Negative (no pigment)	Negative (no pigment)
Nonchromogen (no pigment)	Positive (no pigment)	Positive (no pigment)
Biochemical tests		
Niacin production	Positive	Positive[5,6]
Nitrate reduction	Positive	Positive
Pyrazinamidase	Positive	Positive
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene (~ 320 base pairs)	≥ 99% sequence identity to *M. canetti,* strain CIPT 140060007 (GenBank: AJ749924.1)	100% sequence identity to *M. canetti,* strain CIPT 140060007 (GenBank: AJ749924.1)
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment[7]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar[8]	Report results	Growth consistent with expected colony morphology
Viability (post-freeze)[3]	Growth	Growth

[1]NR-49066 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 30 days at 37°C in an aerobic atmosphere with 5% CO_2 to produce this lot.

[2]Information on Mycobacterium testing is available from Ribón, W. "Biochemical Isolation and Identification of Mycobacteria." Biochemical Testing. (2012) Jose C. Jimenez-Lopez (Ed.), InTech, http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria and Lévy-Frébault, V. V. and F. Portaels. "Proposed Minimal Standards for the Genus *Mycobacterium* and for Description of New Slowly Growing *Mycobacterium* Species." Int. J. Syst. Bacteriol. 42 (1992): 315-323. PubMed: 1581193.

[3]21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment

[4]*M. canettii* produces both smooth and rough phenotypes (Goh, K. S., et al. "Rapid Differentiation of "*Mycobacterium canettii*" from Other *Mycobacterium tuberculosis* Complex Organisms by PCR-Restriction Analysis of the *hsp65* Gene." J. Clin. Microbiol. (2001): 3705-3708. PubMed: 11574597.).

[5]All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.

[6]The niacin specification was established following Vincent, V., et al. "*Mycobacterium*: Phenotypic and Genotypic Identification." In: Murray, P. R., et al. (Eds.), Manual of Clinical Microbiology (8th ed.) Washington, D.C.: ASM Press, pp. 560-584, when *M. canettii* was classified as a subspecies of *M. tuberculosis*. *M. canettii* has since been effectively published, though not validly published, as its own species within the *M. tuberculosis* complex and a niacin production specification has not yet been determined since both positive and negative results have been reported in the literature.

[7]Purity of this lot was assessed for 35 days at 37°C in an aerobic atmosphere with 5% CO_2.

[8]Purity of this lot was assessed for 21 days at 37°C in an aerobic atmosphere with 5% CO_2.

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Figure 1: Colony Morphology

Heather Couch

Digitally signed by Heather Couch
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15 MAR 2018

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