Certificate of Analysis for NR-44267

Mycobacterium intracellulare, Strain 1956

Catalog No. NR-44267

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Product Description:

Mycobacterium intracellulare (M. intracellulare), strain 1956 was isolated in 2011 from human sputum at NIAID, NIH, Bethesda, Maryland, USA. NR-44267 was produced by inoculation of BEI Resources seed lot 62009739 into Middlebrook 7H9 broth with ADC enrichment and grown for 14 days at 37°C in an aerobic atmosphere with 5% CO_2. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 14 days at 37°C in an aerobic atmosphere with 5% CO_2 to produce this lot. Quality control testing was completed under propagation conditions unless otherwise noted.

Lot: 70062659 **Manufacturing Date: 11SEP2023**

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TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[1]		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology	Report results	Circular, convex, entire, smooth and cream (Figure 1)
Motility (wet mount)	Report results	Non-motile
Growth rate	≥ 7 days	14 days
Growth at 45°C	Negative	Negative
Growth at 55°C	Negative	Negative
Acid-fast stain	Positive (red colonies)	Positive (red colonies)
Pigmentation in the dark (Scotochromogen)	Positive (yellow pigment)	Negative (no pigment produced)[2]
Nonchromogen (no pigment)	Negative (yellow pigment)	Positive (no pigment produced)[2]
VITEK® MS (MALDI-TOF)	*M. intracellulare*	*M. intracellulare* (99.9%)
Biochemical tests[3]		
Catalase	Positive	Negative[2]
Catalase (semiquantitative)	Negative	Negative
Catalase (68°C)	Positive	Positive
Iron uptake	Negative	Negative
Nitrate reduction	Negative	Negative
Tween 80	Negative	Negative
Urease	Negative	Negative
Growth in the presence of 5% sodium chloride	Negative	Negative
Growth in the presence of thiophene-2-carboxylic acid hydrazide (TCH)	Positive	Positive
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (1400 base pairs)	≥ 99% sequence identity to *M. intracellulare,* strain 1956 (GenBank: JAOG01000001.1)	100% sequence identity to *M. intracellulare,* strain 1956 (GenBank: JAOG01000001.1)[4]
Sequencing of Heat Shock Protein 65 gene (~ 440 base pairs)	≥ 99% sequence identity to *M. intracellulare,* strain 1956 (GenBank: JAOG01000003.1)	99.8% sequence identity to *M. intracellulare,* strain 1956 (GenBank: JAOG01000003.1)[4]
Digital DNA-DNA hybridization (dDDH)[5]	≥ 70% for species identification	*M. intracellulare* (92.5%)[6] *M. paraintracellulare* (88.6%) *M. indicuspranii* (88.1%) *M. intracellulare* subsp. *chimaera* (78.2%) *M. intracellulare* subsp. *yongonense* (77.8%)

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TEST	SPECIFICATIONS	RESULTS
Purity (post-freeze) Middlebrook 7H10 agar with OADC enrichment[7] 14 days at 37°C in an aerobic atmosphere with 5% CO_2	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Purity (post-freeze) Tryptic Soy agar 14 days at 37°C in an aerobic atmosphere with 5% CO_2	Report results	Growth consistent with expected colony morphology
Viability	Growth	Growth

[1]Information on *Mycobacterium* testing is available from Ribón, W. "Biochemical Isolation and Identification of Mycobacteria." Biochemical Testing. (2012) Jose C. Jimenez-Lopez (Ed.), InTech, Available from: Biochemical Isolation and Identification of Mycobacteria, 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, and Magee, J. G. and A. C. Ward. "Family III. *Mycobacteriaceae* Chester 1897, 63[AL]." Bergey's® Manual of Systematic Bacteriology, Volume 5. (2012) Goodfellow, M., et al. (Ed.), Springer.

[2]The specification for this test was established using the test result from BEI Resources seed lot 62009739. Species-level identification and characterization of *Mycobacterium* spp. through biochemical testing has been shown to produce inconsistent or nonreproducible results. The use of whole genome sequencing (WGS) and digital DNA-DNA hybridization (dDDH) provides quantitative species-level identification that is accurate, precise, and reproducible. BEI Resources will be discontinuing the use of biochemical testing to identify *Mycobacterium* spp. in favor of WGS/dDDH. For more information, refer to Forbes, B. A., et al. "Practical Guidance for Clinical Microbiology Laboratories: Mycobacteria." Clin. Microbiol. Rev. 31 (2018): e00038-17. PubMed: 29386234.

[3]Negative tests are observed for > 7 days.

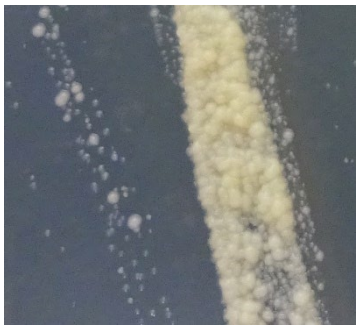
[4]Also consistent with other members of the *M. avium-M. intracellulare* species complex

[5]Relatedness between bacterial strains has traditionally been determined using DDH. For additional information refer to Auch, A.F., et al. "Digital DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." Stand. Genomic Sci. 2 (2010): 117-134. PubMed: 21304684.

[6]The whole genome of *M. intracellulare,* strain 1956 (contig total length approximately 5.44 megabase pairs) was sequenced using the Illumina® NextSeq® system.

[7]M7H10 agar with OADC enrichment contains malachite green, which may inhibit growth of contaminating microorganisms

Figure 1: Colony Morphology



/Sonia Bjorum Brower/
Sonia Bjorum Brower 29 JAN 2025
Technical Manager or designee, ATCC Federal Solutions

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Support Provided by NIAID

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