b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-19028

Mycobacterium tuberculosis, Strain HN4447

Catalog No. NR-19028

Product Description: *Mycobacterium tuberculosis* (*M. tuberculosis*), strain HN4447 was isolated in 2004 from human pulmonary tissue in Texas, USA. Strain HN4447 was deposited as resistant to ethambutol.

Lot[1]: 62886753 **Manufacturing Date: 10OCT2014**

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 (Figure 1[4])
Growth rate	≥ 7 days	18 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[5]	Positive	Positive
Nitrate reduction	Positive	Positive
Pyrazinamidase	Positive	Positive
Antibiotic Susceptibility Profile		
Sensititre™ System[6,7]		
Amikacin	Report results	≤ 0.12 µg/mL
Cycloserine	Report results	= 8 µg/mL
Ethambutol	Report results	= 8 µg/mL
Ethionamide	Report results	= 1.2 µg/mL
Isoniazid	Report results	≤ 0.03 µg/mL
Kanamycin	Report results	≤ 0.6 µg/mL
Moxifloxacin	Report results	≤ 0.06 µg/mL
Ofloxacin	Report results	= 0.5 µg/mL
Para-aminosalicylic acid	Report results	≤ 0.5 µg/mL
Rifabutin	Report results	≤ 0.12 µg/mL
Rifampin	Report results	≤ 0.12 µg/mL
Streptomycin	Report results	≤ 0.25 µg/mL
Genotypic Analysis Sequencing of Heat Shock Protein 65 gene (~ 400 base pairs)	≥ 99% sequence identity to *M. tuberculosis* type strain (GenBank: AL123456)	100% sequence identity to *M. tuberculosis* type strain (GenBank: AL123456)[8]
Purity (post-freeze)[9]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)[4]	Growth	Growth

[1]NR-19028 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment and grown for 27 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 25 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]18 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment

[4]The photograph in Figure 1 shows growth after 28 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment.

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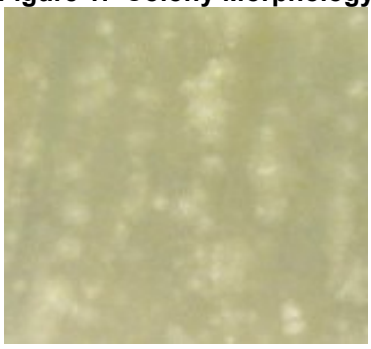
[5]All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.
[6]Sensititre™ System *Mycobacterium tuberculosis* MIC Plate, Thermo Scientific™, catalog number MYCOTB
[7]No interpretations of the Sensititre™ System data for *M. tuberculosis* are currently available.
[8]Also consistent with *M. africanum*, *M. bovis* and *M. microti*
[9]Purity of this lot was assessed for 21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment and on Tryptic Soy agar plates.

Figure 1: Colony Morphology



Date: 01 DEC 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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