Certificate of Analysis for NR-43793

Mycobacterium tuberculosis, Strain KT-0008

Catalog No. NR-43793

Product Description:

Mycobacterium tuberculosis (M. tuberculosis), strain KT-0008 was isolated from a human in South Korea. Strain KT-0008 was deposited as a multidrug-resistant (MDR) Beijing genotype strain with resistance to capreomycin, isoniazid, kanamycin, pyrazinamide and rifampin. NR-43793 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 44 days at 37°C in an aerobic atmosphere with 5% CO_2 to produce this lot.

Lot: 70013977 **Manufacturing Date: 01JUN2018**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[1]		
Cellular morphology 21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment	Gram-positive rods	Gram-positive rods
Colony morphology	Report results	Irregular, slight peaked, undulate, rough and cream (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[2]	Positive	Positive
Nitrate reduction	Positive	Positive
Antibiotic Susceptibility Profile		
Sensititre™ System[3,4]		
Amikacin	Report results	16 µg/mL
Cycloserine	Report results	64 µg/mL[5,6]
Ethambutol	Report results	4 µg/mL[6,7,8]
Ethionamide	Report results	2.5 µg/mL[7]
Isoniazid	Report results	1 µg/mL[6,9]
Kanamycin	Report results	> 40 µg/mL
Moxifloxacin	Report results	0.25 µg/mL
Ofloxacin	Report results	2 µg/mL[6,10]
Para-aminosalicylic acid	Report results	2 µg/mL[6,7,11]
Rifabutin	Report results	0.5 µg/mL[7]
Rifampin	Report results	> 16 µg/mL[6,12]
Streptomycin	Report results	0.5 µg/mL[7]
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene (~ 1620 base pairs)	≥ 99% sequence identity to *M. tuberculosis,* strain KT-0008 (GenBank: JLSJ01000013.1)	100% sequence identity to *M. tuberculosis,* strain KT-0008 (GenBank: JLSJ01000013.1)[13]
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment 32 days at 37°C in an aerobic atmosphere with 5% CO_2	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar 21 days at 37°C in an aerobic atmosphere with 5% CO_2	Report results	Growth consistent with expected colony morphology

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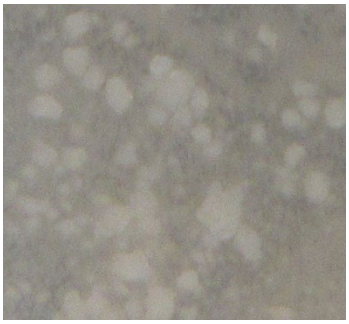
TEST	SPECIFICATIONS	RESULTS
Viability (post-freeze) 21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment	Growth	Growth

[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, 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.
[2]All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.
[3]Sensititre™ System *Mycobacterium tuberculosis* MIC Plate, Thermo Scientific™, catalog number MYCOTB
[4]Minimum Inhibitory Concentration (MIC); No Clinical & Laboratory Standards Institute (CLSI) interpretations of the Sensititre™ System data for *M. tuberculosis* are currently available.
[5]Two MICs were observed for cycloserine (32 µg/mL and 64 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.
[6]Variability in the MIC result by the Sensititre™ method has been demonstrated [Lee, J., et al. "Sensititre MYCOTB MIC Plate for Testing *Mycobacterium tuberculosis* Susceptibility to First- and Second-Line Drugs." Antimicrob. Agents Chemother. 58 (2014): 11-18. PubMed: 24100497.], with the results for a single antibiotic typically within one doubling dilution.
[7]For ethambutol, ethionamide, para-aminosalicylic acid, rifabutin and streptomycin, the endpoint for these drugs is determined by the well with approximately 80% inhibition of growth compared to the positive control well with no drug.
[8]Two MICs were observed for ethambutol (2 µg/mL and 4 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.
[9]Two MICs were observed for isoniazid (0.5 µg/mL and 1 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.
[10]Two MICs were observed for ofloxacin (1 µg/mL and 2 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.
[11]Two MICs were observed for para-aminosalicylic acid (1 µg/mL and 2 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.
[12]Two MICs were observed for rifampin (16 µg/mL and > 16 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.
[13]Also consistent with *M. africanum*, *M. bovis*, *M. canettii*, *M. caprae* and *M. microti*

Figure 1: Colony Morphology



/Heather Couch/
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