Certificate of Analysis for NR-43802

Mycobacterium tuberculosis, Strain KT-0017

Catalog No. NR-43802

Product Description:

Mycobacterium tuberculosis (M. tuberculosis), strain KT-0017 was isolated from a human in South Korea. Strain KT-0017 was deposited as an extensively drug-resistant (XDR) Beijing genotype strain, with resistance to capreomycin, isoniazid, kanamycin, moxifloxacin, ofloxacin, pyrazinamide, rifampin and streptomycin. NR-43802 was produced by inoculation of the deposited material into Middlebrook 7H9 broth with ADC enrichment. After one passage, the culture was grown for 21 days in an aerobic atmosphere with 5% CO_2 in Middlebrook 7H9 broth with ADC enrichment. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 19 days at 37°C in an aerobic atmosphere with 5% CO_2 to produce this lot.

Lot: 70014683 **Manufacturing Date: 29AUG2018**

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
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	16 µg/mL
Ethambutol	Report results	8 µg/mL[5]
Ethionamide	Report results	20 µg/mL[5,6,7]
Isoniazid	Report results	4 µg/mL
Kanamycin	Report results	> 40 µg/mL
Moxifloxacin	Report results	8 µg/mL
Ofloxacin	Report results	32 µg/mL[6,8]
Para-aminosalicylic acid	Report results	> 64 µg/mL[5]
Rifabutin	Report results	8 µg/mL[5]
Rifampin	Report results	> 16 µg/mL
Streptomycin	Report results	> 32 µg/mL[5]
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene (~ 1620 base pairs)	≥ 99% sequence identity to *M. tuberculosis*, strain KT-0017 (GenBank: JLSE01000019.1)	99.9% sequence identity to *M. tuberculosis*, strain KT-0017 (GenBank: JLSE01000019.1)[9]
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment 44 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]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.

[6]Two MICs were observed for ethionamide (10 µg/mL and 20 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.

[7]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.

[8]Two MICs were observed for ofloxacin (16 µg/mL and 32 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.

[9]Also consistent with *M. africanum*, *M. bovis*, *M. canettii*, *M. caprae* and *M. microti*

/Heather Couch/
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