

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 ciprofloxacin, 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% CO₂ 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% CO₂ to produce this lot.

Lot: 70014683 Manufacturing Date: 29AUG2018

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ¹		
Cellular morphology	Gram-positive rods	Gram-positive rods
21 days at 37°C in an aerobic atmosphere with 5% CO ₂		
on Middlebrook 7H10 agar with OADC enrichment		
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	≥ 99% sequence identity to	99.9% sequence identity to
(~ 1620 base pairs)	M. tuberculosis, strain KT-0017(GenBank: JLSE01000019.1)	M. tuberculosis, strain KT-001 (GenBank: JLSE01000019.1)⁹
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment	Growth consistent with expected	Growth consistent with expected
44 days at 37°C in an aerobic atmosphere with 5% CO ₂	colony morphology	colony morphology
Tryptic Soy agar	Report results	Growth consistent with expected
21 days at 37°C in an aerobic atmosphere with 5% CO ₂		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

Information on Mycobacterium testing is available from Ribón, W. "Biochemical Isolation and Identification of Mycobacteria." <u>Biochemical Testing.</u> (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." <a href="https://link.doi.org/10.1008/jns.

²All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.

³Sensititre™ System Mycobacterium tuberculosis MIC Plate, Thermo Scientific™, catalog number MYCOTB

/Heather Couch/

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Program Manager or designee, ATCC Federal Solutions

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⁴Minimum Inhibitory Concentration (MIC); No Clinical & Laboratory Standards Institute (CLSI) interpretations of the Sensititre™ System data for *M. tuberculosis* are currently available.

⁵For 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.

⁶Two 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.

⁷Variability 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." <u>Antimicrob. Agents Chemother.</u> 58 (2014): 11-18. PubMed: 24100497.], with the results for a single antibiotic typically within one doubling dilution.

⁸Two 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. ⁹Also consistent with *M. africanum*, *M. bovis*, *M. canettii*, *M. caprae* and *M. microti*