SUPPORTING INFECTIOUS DISEASE RESEARCH

Mycobacterium tuberculosis, Strain KT-0016

Catalog No. NR-43801

Product Description:

Mycobacterium tuberculosis (M. tuberculosis), strain KT-0016 was isolated from a human in South Korea. Strain KT-0016 was deposited as an extensively drug-resistant (XDR) Beijing genotype strain with resistance to capreomycin, isoniazid, kanamycin, moxifloxacin, ofloxacin, pyrazinamide, rifampin and streptomycin. NR-43801 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 38 days at 37°C in an aerobic atmosphere with 5% CO₂ to produce this lot.

Lot: 70014681

Manufacturing Date: 01JUN2018

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,
	Report results	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)
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	8 μg/mL ⁷
Ethionamide	Report results	20 μg/mL ^{6,7,8}
Isoniazid	Report results	> 4 μg/mL ^{6,9}
Kanamycin	Report results	> 40 μg/mL
Moxifloxacin	Report results	8 μg/mL
Ofloxacin	Report results	16 μg/mL
Para-aminosalicylic acid	Report results	> 64 μg/mL ⁷
Rifabutin	Report results	1 μg/mL ⁷
Rifampin	Report results	> 16 µg/mL
Streptomycin	Report results	1 μg/mL ^{6,7,10}
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene	≥ 99% sequence identity to	100% sequence identity to
(~ 1620 base pairs)	<i>M. tuberculosis</i> , strain KT-0016	<i>M. tuberculosis</i> , strain KT-0016
	(GenBank: JLSF01000011.1)	(GenBank: JLSF01000011.1) ¹¹
Purity (post-freeze)		Crowth consistent with sum ()
Middlebrook 7H10 agar with OADC enrichment 45 days at 37°C in an aerobic atmosphere with 5% CO ₂	Growth consistent with expected	Growth consistent with expected
Tryptic Soy agar	colony morphology Report results	colony morphology Growth consistent with expected
21 days at 37°C in an aerobic atmosphere with 5% CO ₂		colony morphology
$2 + 4 a y_3 a t_0 + 0$ in an actionic autosphere with $3 / 0 + 0 0 2$		colorry morphology

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Certificate of Analysis for NR-43801

SUPPORTING INFECTIOUS DISEASE RESEARCH

TEST	SPECIFICATIONS	RESULTS
Viability (post-freeze)	Growth	Growth
21 days at 37°C in an aerobic atmosphere with 5% CO ₂		
on Middlebrook 7H10 agar with OADC enrichment		

¹Information 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, <u>http://www.intechopen.com/books/biochemical-testing/biochemical-isolation-and-identification-of-mycobacteria</u> 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.

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

³SensititreTM System Mycobacterium tuberculosis MIC Plate, Thermo ScientificTM, catalog number MYCOTB

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

⁵Two 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.
⁶Variability 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.

⁷For 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.

⁸Two 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.

⁹Two MICs were observed for isoniazid (4 μg/mL and > 4 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.
¹⁰Two MICs were observed for streptomycin (0.5 μg/mL and 1 μg/mL) under identical test conditions. The highest MIC is being reported as the test result.

¹¹Also consistent with *M. africanum*, *M. bovis*, *M. canettii*, *M. caprae* and *M. microti*

Figure 1: Colony Morphology



/Heather Couch/ Heather Couch

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