b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49376

Mycobacterium tuberculosis, Strain XTB13-250

Catalog No. NR-49376

Product Description: *Mycobacterium tuberculosis (M. tuberculosis)*, strain XTB13-250 was isolated in 2012 from the sputum of a patient with tuberculosis in the Republic of Belarus. Strain XTB13-250 was deposited as resistant to ethambutol, isoniazid, ofloxacin, pyrazinamide, rifampin and streptomycin.

Lot[1]: 64064242 **Manufacturing Date: 12MAY2016**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis[2]		
Cellular morphology	Gram-positive rods	Gram-positive rods
Colony morphology[3]	Report results	Irregular, low convex, 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[4]	Positive	Positive
Nitrate reduction	Positive	Positive
Pyrazinamidase	Positive	Positive
Antibiotic Susceptibility Profile		
Sensititre™ System[5,6]		
Amikacin	Report results	1 µg/mL
Cycloserine	Report results	16 µg/mL
Ethambutol	Report results	16 µg/mL[7,8,9]
Ethionamide	Report results	> 40 µg/mL[7,9,10]
Isoniazid	Report results	> 4 µg/mL
Kanamycin	Report results	2.5 µg/mL
Moxifloxacin	Report results	4 µg/mL
Ofloxacin	Report results	8 µg/mL
Para-aminosalicylic acid	Report results	4 µg/mL[7,9,11]
Rifabutin	Report results	8 µg/mL[7]
Rifampin	Report results	> 16 µg/mL
Streptomycin	Report results	> 32 µg/mL[7]
Genotypic Analysis		
Sequencing of Heat Shock Protein 65 gene (~ 440 base pairs)	≥ 99% sequence identity to *M. tuberculosis*, strain XTB13-250 (GenBank: JLHG01000002.1)	100% sequence identity to *M. tuberculosis*, strain XTB13-250 (GenBank: JLHG01000002.1)[12]
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment[13]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Tryptic Soy agar[14]	Report results	No growth
Viability (post-freeze)[3]	Growth	Growth

[1]NR-49376 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 63 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-

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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]21 days at 37°C in an aerobic atmosphere with 5% CO_2 on Middlebrook 7H10 agar with OADC enrichment

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

[5]Sensititre™ System *Mycobacterium tuberculosis* MIC Plate, Thermo Scientific™, catalog number MYCOTB

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

[7]For streptomycin, ethionamide, para-aminosalicylic acid, rifabutin and ethambutol, 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]Three MICs were observed for ethambutol (4 µg/mL, 8 µg/mL and 16 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.

[9]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.

[10]Three MICs were observed for ethionamide (20 µg/mL, 40 µg/mL and > 40 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.

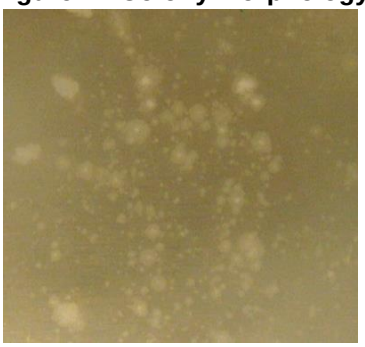
[11]Three MICs were observed for para-aminosalicylic acid (1 µg/mL, 2 µg/mL and 4 µg/mL) under identical test conditions. The highest MIC is being reported as the test result.

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

[13]Purity of this lot was assessed for 50 days at 37°C in an aerobic atmosphere with 5% CO_2.

[14]Purity of this lot was assessed for 21 days at 37°C in an aerobic atmosphere with 5% CO_2.

Figure 1: Colony Morphology



Heather Couch

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