Certificate of Analysis for NR-122

Mycobacterium tuberculosis subsp. *tuberculosis*, Strain H37Ra

Catalog No. NR-122
(Derived from ATCC® 25177™)

Product Description:
Mycobacterium tuberculosis (M. tuberculosis) subsp. *tuberculosis*, strain H37Ra is an attenuated strain derived from the virulent parent strain H37. Strain H37 was isolated in 1905 from the sputum of a patient with chronic pulmonary tuberculosis. NR-122 was produced by inoculation of BEI Resources NR-122 lot 3685764 into Middlebrook 7H9 broth with ADC enrichment. Broth inoculum was added to Middlebrook 7H10 agar with OADC enrichment kolles, which were grown for 21 days at 37°C in an aerobic atmosphere to produce this lot.

Lot: 70030637 **Manufacturing Date: 12NOV2019**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology 14 days at 37°C in an aerobic atmosphere on Middlebrook 7H10 agar with OADC enrichment	Gram-positive rod	Gram-positive rod
Colony morphology	Report results	Irregular, convex, undulate, rough and cream (Figure 1)
Growth on Brain Heart Infusion agar[1]	Report results	Growth
Growth on MacConkey agar (without crystal violet)[1]	No growth	No growth
Motility	Non-motile	Non-motile
Growth rate	≥ 7 days	21 days
Growth at 26°C[1]	Negative	Negative
Growth at 37°C	Positive	Positive
Growth at 46°C[1]	Report results	No growth
Growth at 55°C[1]	Report results	No growth
Acid-fast stain	Positive (Red colonies)	Positive (Red colonies)
Pigmentation in the dark (Scotochromogen)[1]	Negative (no pigment)	Negative (no pigment)
Photoinduction for 1 hour (Photochromogen)[1]	Negative (no pigment)	Negative (no pigment)
Nonchromogen (no pigment)[1]	Positive (no pigment)	Positive (no pigment)
Biochemical tests		
Niacin production[2]	Positive	Positive
Nitrate reduction[1]	Positive	Positive
Pyrazinamidase[1]	Positive	Positive
Urease[1]	Positive	Positive
Aryl sulfate (3 days)[1]	Negative	Negative
Aryl sulfate (14 days)[1]	Negative	Negative
Catalase (semiquantitative)[1]	Negative	Negative
Catalase (68°C, pH 7)[1]	Negative	Negative
Iron uptake[1]	Negative	Negative
Tween 80 hydrolysis[1]	Report results	Positive
Growth in the presence of 5% sodium chloride[1,3]	Report results	Negative
Growth in the presence of thiophene-2 carboxylic acid hydrazide (TCH)[1,4]	Report results	Positive
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1430 base pairs)	≥ 99% sequence identity to *M. tuberculosis,* strain H37Ra (GenBank: CP000611.1)	100% sequence identity to *M. tuberculosis,* strain H37Ra (GenBank: CP000611.1)[5]
Sequencing of Heat Shock Protein 65 gene (~ 430 base pairs)	≥ 99% sequence identity to *M. tuberculosis,* strain H37Ra (GenBank: CP000611.1)	100% sequence identity to *M. tuberculosis,* strain H37Ra (GenBank: CP000611.1)[5]

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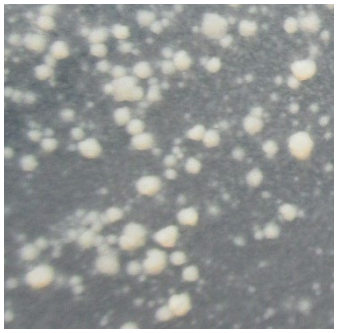
TEST	SPECIFICATIONS	RESULTS
Purity (post-freeze)		
Middlebrook 7H10 agar with OADC enrichment 21 days at 37°C in an aerobic atmosphere	Growth consistent with expected colony morphology Report results	Growth consistent with expected colony morphology Growth consistent with expected colony morphology
Tryptic Soy agar 21 days at 37°C in an aerobic atmosphere with and without 5% CO_2		
Viability (post-freeze) 14 days at 37°C in an aerobic atmosphere on Middlebrook 7H10 agar with OADC enrichment	Growth	Growth

[1]Performed on BEI Resources NR-122 lot 3685764
[2]All mycobacteria produce niacin but only *M. tuberculosis* accumulates it, resulting in a positive test for *M. tuberculosis*.
[3]85% of *M. tuberculosis* strains are positive
[4]Less than 15% of *M. tuberculosis* strains are positive.
[5]Also consistent with *M. africanum*, *M. bovis*, *M. canettii*, *M. caprae* and *M. microti*

Figure 1: Colony Morphology



/Heather Couch/
Heather Couch 17 SEP 2020
Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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