

Certificate of Analysis for NR-43658

Helicobacter pylori, Strain Hp A-20

Catalog No. NR-43658

Product Description: *Helicobacter pylori* (*H. pylori*), strain Hp A-20 was isolated from gastric biopsy homogenate of a patient with a duodenal ulcer in Ohio, USA.

Lot¹: 64136548 Manufacturing Date: 22APR2016

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology ²	Report results	Circular, low convex, entire, translucent and gray (Figure 1)
Motility (wet mount) Biochemical characterization	Report results	Motile
Catalase	Positive	Positive
Oxidase	Positive	Positive
Urease	Positive	Positive
Nitrate reduction	Negative	Negative
H2S (lead acetate paper)	Report results	Positive
Hippurate hydrolysis	Negative	Negative
Growth with 5% CO ₂	Growth	Growth
Growth at 25°C	No growth	No growth
Growth at 37°C	Growth	Growth
Growth at 42°C	Report results	No growth
Brucella albimi + 0.16% agar (growth control)	Growth	Growth
Brucella albimi + 0.16% agar with 1% glycine	No growth	No growth ³
Brucella albimi + 0.16% agar with 1.% glycine Brucella albimi + 0.16% agar with 3.5% NaCl	No growth	No growth ⁴
Antibiotic Susceptibility Profile BD BBL™ Sensi-Disc™ susceptibility test discs Metronidazole (80 µg) ⁵ Nalidixic acid (30 µg) ⁶	Report results Report results	50 mm 9 mm
ivalidixic acid (50 µg) ^s	Report results	9 111111
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1390 base pairs)	≥ 99% sequence identity to H. pylori, strain Hp A-20 (GenBank: AKOE01000006.1)	100% sequence identity to H. pylori, strain Hp A-20 (GenBank: AKOE01000006.1)
Confirmation of <i>H. pylori</i> by PCR Amplification of		
Extracted DNA		
Positive control (16S ribosomal RNA gene)	Amplicon present	Amplicon present
Negative control (H. acinonychis)	No amplicon present	No amplicon present
ureA	Amplicon present	Amplicon present
ssaA	Amplicon present	Amplicon present
Purity (post-freeze)		
Microaerophilic growth ⁷	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Aerobic growth ^{8,9}	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze) ²	Growth	Growth

NR-43658 was produced by inoculation of the deposited material into Brucella broth. Broth inoculum was added to Columbia agar with 7% defibrinated horse blood, 5 μg/mL trimethoprim, 5 μg/mL vancomycin, 10 μg/mL cefsulodin and 2.5 μg/mL amphotericin B. The inoculated agar and broth were each grown for 5 days at 37°C in a microaerophilic atmosphere (~ 6-16% O₂ and 2-10% CO₂). Colonies from the Columbia agar culture were suspended into the Brucella broth growth, and this biphasic culture was added to Columbia agar with 7% defibrinated horse blood, 5 μg/mL trimethoprim, 5 μg/mL vancomycin, 10 μg/mL cefsulodin and 2.5 μg/mL amphotericin B kolles, which were grown for 4 days at 37°C in a microaerophilic atmosphere to produce this lot.

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Figure 1: Colony Morphology

Date: 28 SEP 2016

Signature:

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²3 days on Columbia agar with 7% defibrinated horse blood, 5 μg/mL trimethoprim, 5 μg/mL vancomycin, 10 μg/mL cefsulodin and 2.5 μg/mL amphotericin B at 37°C in a microaerophilic atmosphere

³Specifications for these tests were obtained from Bergey's Manual[®] of Systematic Bacteriology, 2nd ed., Volume 2, Part C, which indicates that growth may occur in up to 17% of strains.

⁴Specifications for these tests were obtained from Bergey's Manual® of Systematic Bacteriology, 2nd ed., Volume 2, Part C, which indicates that growth may occur in 20% to 43% of strains.

⁵Test performed using metronidazole 80 μg (MET-80, BBI™ catalog no. 231605)

⁶Test performed using nalidixic acid 30 µg (NA-30, BBL™ catalog no. 231311)

⁷Purity of this lot was assessed for 3 days on Tryptic Soy agar with 5% defibrinated sheep blood at 37°C in a microaerophilic atmosphere (~ 6-16% O₂ and 2-10% CO₂).

⁸Purity of this lot was assessed for 7 days on Tryptic Soy agar with 5% defibrinated sheep blood at 37°C in an aerobic atmosphere with 5% CO₂. ⁹H. pylori is known to show weak growth under aerobic conditions (Bury-Moné, S., et al. "Is Helicobacter pylori a True Microaerophile?" Helicobacter 11 (2006): 296-303. PubMed: 16882333.).