# Certificate of Analysis for NR-43654

## *Helicobacter pylori*, Strain Hp A-5

## Catalog No. NR-43654

**Product Description:** *Helicobacter pylori* (*H. pylori*), strain Hp A-5 was isolated from gastric biopsy homogenate of a patient with a gastric ulcer in Ohio, USA.

**Lot[1]: 64136547** **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) | Report results | Motile |
| Biochemical characterization | | |
| 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 3.5% NaCl | No growth | No growth[4] |
| **Antibiotic Susceptibility Profile** | | |
| BD BBL™ Sensi-Disc™ susceptibility test discs | | |
| Metronidazole (80 µg)[5] | Report results | 49 mm |
| Nalidixic acid (30 µg)[6] | Report results | 8 mm |
| **Genotypic Analysis** | | |
| Sequencing of 16S ribosomal RNA gene (~ 1410 base pairs) | ≥ 99% sequence identity to *H. pylori,* strain Hp A-5 (GenBank: AKOB01000005.1) | 100% sequence identity to *H. pylori,* strain Hp A-5 (GenBank: AKOB01000005.1) |
| **Confirmation of *H. pylori* 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 |

[1]NR-43654 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% $O_2$ and 2-10% $CO_2$). 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.

[2]3 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

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[3]Specifications 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.
[4]Specifications 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.
[5]Test performed using metronidazole 80 µg (MET-80, BBI™ catalog no. 231605)
[6]Test performed using nalidixic acid 30 µg (NA-30, BBL™ catalog no. 231311)
[7]Purity 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% $O_2$ and 2-10% $CO_2$).
[8]Purity 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% $CO_2$.
[9]*H. 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.).

**Figure 1: Colony Morphology**

**Date:** 28 SEP 2016 **Signature:**

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