Certificate of Analysis for NR-43673

Helicobacter pylori, Strain Hp A-14

Catalog No. NR-43673

Product Description: *Helicobacter pylori* (*H. pylori*), strain Hp A-14 was isolated from gastric biopsy homogenate of a patient with gastritis in Ohio, USA.

Lot[1]: 64136571 **Manufacturing Date: 15APR2016**

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	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	53 mm
Nalidixic acid (30 µg)[6]	Report results	8 mm
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1390 base pairs)	≥ 99% sequence identity to *H. pylori* type strain (GenBank: AIHX01000070.1)	99.7% sequence identity to *H. pylori* type strain (GenBank: AIHX01000070.1)
Digital DNA-DNA hybridization (dDDH)[7]	> 70% agreement for species identification	*H. pylori* (91.8%)
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	No amplicon present[8]
ssaA	Amplicon present	Amplicon present
Purity (post-freeze)		
Microaerophilic growth[9]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Aerobic growth[10,11]	Growth consistent with expected colony morphology	Growth consistent with expected colony morphology
Viability (post-freeze)[2]	Growth	Growth

[1]NR-43673 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 4 days at 37°C in a microaerophilic atmosphere (~ 6-16% O_2 and 2-10% CO_2). Colonies from the Columbia agar

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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 37°C in a microaerophilic atmosphere to produce this lot.

[2]7 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

[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, BBL™ catalog no. 231605)

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

[7]Relatedness between bacterial strains has traditionally been determined using DDH. For additional information refer to Auch, A.F., et al. "Digital DNA-DNA Hybridization for Microbial Species Delineation by Means of Genome-to-Genome Sequence Comparison." Stand Genomic Sci, 2 (2010): 117-134, PubMed: 21304684.

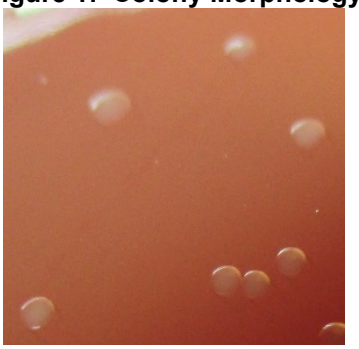
[8]NR-43673 failed to produce the ~380 base pair amplicon corresponding to the *H. pylori*-specific *ureA* gene. Sequence analysis of the *ureA* gene from *H. pylori*, Strain Hp A-14 (GenBank: AKOT00000000.1) identified sequence differences between the type strain on which the primers were based and the sequence of strain Hp A-14, in the binding site for the reverse primer, which negatively affected extension from this primer and resulted in no amplicon being generated.

[9]Purity of this lot was assessed for 7 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).

[10]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.

[11]*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: 26 OCT 2016 **Signature:**

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