

## **Certificate of Analysis for NR-19891**

## Leptospira interrogans, Strain HAI0156 (Serovar Copenhageni)

Catalog No. NR-19891

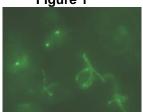
**Product Description:** Leptospira interrogans (L. interrogans), strain HAI0156 (serovar Copenhageni) is a human isolate obtained from a patient at Hospital de Apoyo in Iquitos, Peru.

Lot<sup>1</sup>: 62710998 Manufacturing Date: 15SEP2014

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Spirochete	Spirochete
Colony morphology	Growth below the soft agar surface (Dinger's disk)	Growth below the soft agar surface (Dinger's disk) <sup>2</sup>
Motility (wet mount)	Motile	Motile
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 850 base pairs)	Consistent with L. interrogans	Consistent with <i>L. interrogans</i> <sup>3</sup>
Purity (post-freeze) <sup>4</sup>	No growth	Growth observed <sup>5</sup>
Viability (post-vialing) Visual observation LIVE/DEAD <sup>®</sup> BacLight <sup>™</sup> Bacterial Viability	Growth Green fluorescence visible	Growth <sup>2</sup> Green fluorescence visible (Figure 1) <sup>6</sup>

<sup>&</sup>lt;sup>1</sup>NR-19891 was produced by inoculation of the deposited material into Ellinghausen-McCullough-Johnson-Harrison (EMJH) semisolid agar (0.15%) for 2 days at 30°C in an aerobic atmosphere, and the resulting subculture was vialed and frozen. NR-19891 was produced by inoculation of the frozen subculture into EMJH semisolid agar (0.15%) and grown 32 days at 30°C in an aerobic atmosphere. The material from the initial growth was passaged once in EMJH semisolid agar (0.15%) for 38 days under propagation conditions to produce this lot.

Figure 1



Date: 15 DEC 2014 Signature:

**Title:** Technical Manager, BEI Authentication or designee

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<sup>&</sup>lt;sup>2</sup>Disk of dense growth below the soft agar surface (Dinger's disk) (Czekalowski, J. W., J. W. McLeod and J. Rodican. "The Growth and Respiration of *Leptospira* in Solid or Semi-Solid Media with Special Reference to Dinger's Phenomenon." <u>Br. J. Exp. Pathol.</u> 34 (1953): 588-595.) was evident after 12 days at 30°C in EMJH semisolid agar (0.15%).

<sup>&</sup>lt;sup>3</sup>100% identical to *L. interrogans*, strain HAI0156 (GenBank: AFLP01000082.1)

<sup>&</sup>lt;sup>4</sup>Purity of this lot was assessed for 7 days at 37°C in an aerobic atmosphere on Tryptic Soy agar with 5% defibrinated sheep blood.

<sup>&</sup>lt;sup>5</sup>Unexpected growth was observed. Sequencing of the 16S ribosomal RNA gene from the growth confirmed that it was consistent with *L. interrogans* [100% identical to *L. interrogans*, strain HAl0156 (GenBank: AFLP01000082.1)]

<sup>&</sup>lt;sup>6</sup>Determined after 13 days incubation under cultivation conditions with LIVE/DEAD<sup>®</sup> BacLight<sup>™</sup> Bacterial Viability Kit, 100x magnification (Invitrogen™ L34856). Cells with a compromised membrane that are dead or dying will stain red, while cells with an intact membrane will stain green.