

Certificate of Analysis for NR-35360

Leptospira hovindhougenii, Strain B5-022 (Serovar Osterbro)

Catalog No. NR-35360

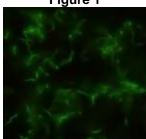
Product Description: Leptospira hovindhougenii (L. hovindhougenii), strain B5-022 (serovar Osterbro) was isolated in Denmark from a Norway rat (*Rattus norvegicus*).

Lot¹: 62380314 Manufacturing Date: 12MAR2014

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) ²
Motility (wet mount)	Motile	Motile
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1450 base pairs)	Consistent with Leptospira spp.	Consistent with <i>Leptospira</i> spp. ³
Viability (post-vialing) Visual observation LIVE/DEAD [®] <i>Bac</i> Light [™] Bacterial Viability	Growth Green fluorescence visible	Growth ² Green fluorescence visible (Figure 1) ⁴

¹NR-35360 was produced by inoculation of the deposited material into Ellinghausen-McCullough-Johnson-Harrison (EMJH) semisolid agar (0.15%) and incubated for 15 days at 30°C in an aerobic atmosphere. The material from the initial growth was passaged once in EMJH semisolid agar (0.15%) for 6 days at 30°C in an aerobic atmosphere to produce this lot. 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% CO₂.

Figure 1



Date: 17 JUN 2014

Signature:

Title: Technical Manager, BEI Authentication or designee

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² 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 7 days at 30°C in EMJH semisolid agar (0.15%).

³≥ 99% identical to *Leptospira sp*, strain B5-022 (GenBank: ANIJ01000006.1)

⁴Determined after 7 days incubation under cultivation conditions with LIVE/DEAD[®] BacLight[™] 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.