

Certificate of Analysis for NR-22253

Leptospira broomii, Strain 5399T (Serovar Hurstbridge)

Catalog No. NR-22253

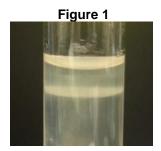
Product Description: Leptospira broomii (L. broomii), strain 5399T (serovar Hurstbridge) is an intermediately pathogenic strain isolated from the blood of a human with acute leptospirosis in Denmark.

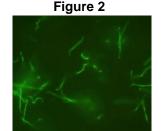
Lot¹: 62359962 Manufacturing Date: 20MAR2014

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 (Figure 1)
Motility (wet mount)	Motile	Motile
Genotypic Analysis		
Sequencing of 16S ribosomal RNA gene (~ 1370 base pairs)	Consistent with L. broomii	Consistent with <i>L. broomii</i> ^{3,4}
Viability (post-vialing)		
Visual observation	Growth	Growth ²
LIVE/DEAD [®] <i>Bac</i> Light [™] Bacterial Viability ⁵	Green fluorescence visible	Green fluorescence visible (Figure 2) ⁵

¹The deposited material was inoculated into Ellinghausen-McCullough-Johnson-Harrison (EMJH) semisolid agar (0.15%) and grown 17 days at 30°C in an aerobic atmosphere, and the resulting growth was vialed and frozen. NR-22253 was produced by inoculation of the frozen subculture into EMJH semisolid agar (0.15%) and incubated for 22 days at 30°C in an aerobic atmosphere. The material from the second growth was passaged in EMJH semisolid agar (0.15%) for 8 days at 30°C in an aerobic atmosphere to produce this lot. Purity of this lot was assessed for 22 days on Tryptic Soy agar with 5% defibrinated sheep blood at 37°C in an aerobic atmosphere with 5% CO₂.

⁵Determined after 65 days incubation under cultivation conditions with LIVE/DEAD[®] *Bac*Light[™] 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.





Date: 26 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 65 days at 30°C in EMJH semisolid agar (0.15%).

³Also consistent with other *Leptospira* species

⁴≥ 99% identical to *L. broomii*, strain 5399T (GenBank: AHMO02000008.1)