Certificate of Analysis for NR-46509

Naegleria fowleri, Strain CDC:V636

Catalog No. NR-46509

This reagent is the tangible property of the U.S. Government.

Product Description: *Naegleria fowleri (N. fowleri)*, strain CDC:V636 is a clinical isolate collected in 2012 from the cerebrospinal fluid of an 8-year-old male.

Lot[1]: 70000225 **Manufacturing Date: 22NOV2016**

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology[2]	Report results	Refractile and motile
Genotyping[3] Sequencing of Internal Transcribed Spacer 1 (ITS), 5.8S ribosomal RNA (rRNA) gene, ITS 2 (~ 550 base pairs)	Consistent with *N. fowleri*	Consistent with *N. fowleri* (genotype I)[4,5]
Functional Activity by PCR Amplification[3,6] ITS 1, 5.8S rRNA gene	~ 600 base pair amplicon	~ 600 base pair amplicon
Viable Cell Count by Hemacytometry[3]	> 10^6 cells/mL	9.6 x 10^6 cells/mL
Viability[2,7]	Growth	Growth
Sterility (21-day incubation)[2] Harpo's HTYE broth[8], 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	 No growth No growth No growth No growth No growth No growth No growth	 No growth No growth No growth No growth No growth No growth No growth

[1]NR-46509 was produced by cultivation of the deposited material in modified PYNFH medium (ATCC® medium 1034) supplemented with 10% heat-inactivated fetal bovine serum for 1 day at 35°C in an aerobic atmosphere until peak density was reached.
[2]Testing completed on vialed, post-freeze material.
[3]Testing completed on bulk material prior to vialing and freezing.
[4]For genotyping details refer to Zhou, L., et al. "Genetic Variations in the Internal Transcribed Spacer and Mitochondrial Small Subunit rRNA Gene of *Naegleria* Spp." J. Eukaryot. Microbiol. 50 (2003): 522-526. PubMed: 14736150.
[5]Also consistent with *Naegleria lovaniensis*
[6]PCR amplification was performed using the NF-ITS-F1 and NT-ITS-F2 primer set as described in Zhou, L., et al. "Genetic Variations in the Internal Transcribed Spacer and Mitochondrial Small Subunit rRNA Gene of *Naegleria* Spp." J. Eukaryot. Microbiol. 50 (2003): 522-526. PubMed: 14736150.
[7]Viable cells were observed after 1 day under cultivation conditions.
[8]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 21 JUN 2017 **Signature:**

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