

Certificate of Analysis for NR-59701

Treponema pallidum subsp. pallidum, Strain Nichols (Houston) (in vitro)

Catalog No. NR-59701

Product Description:

Treponema pallidum (T. pallidum) subsp. pallidum, strain Nichols (Houston) was deposited to BEI Resources as a clone of strain Nichols, adapted to *in vitro* culture in *Sylvilagus floridanus* (cottontail rabbit) epithelial cells (ATCC® CCL-68 $^{\text{TM}}$). Strain Nichols (Houston) is a continually propagated laboratory clone of strain Nichols established in 1982. The parental strain Nichols was originally isolated in 1912 from the cerebrospinal fluid of a human subject with recurrent neurosyphilis following treatment with neosalvarsan in Washington, D.C., USA. NR-59701 was produced by inoculation of the testes of a New Zealand white rabbit with a frozen stock from a prior rabbit infection. On day 10 post-infection, the animal was euthanized, and the testes were immediately aseptically removed and minced. NR-59701 was extracted from the minced tissue in *T. pallidum* Cultivation Medium 2 (TpCM-2) with 20% heat-inactivated fetal bovine serum (FBS) under microaerobic conditions (1.5% O₂) for 30 minutes followed by centrifugation twice at 500 × g for 7 minutes to remove tissue debris.

Lot: 70065867 Manufacturing Date: 25APR2024

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis ¹		
Cellular morphology	Spirochete	Spirochete
Motility (wet mount)	Motile	Motile ²
Concentration ¹	Report results	6.9 × 10 ⁷ cells/mL
Amount per vial ¹	Report results	8.6 × 10 ⁷ cells in 1.25 mL
Viability (post-freeze) ¹	Growth	Growth ³

¹Production and QC testing were performed by the depositor [Steven J. Norris, Ph.D., Professor and Vice Chair for Research, Department of Pathology and Laboratory Medicine, University of Texas Health Science Center at Houston McGovern Medical School, Houston, Texas, USA].

²Motility was confirmed by examination by darkfield microscopy following the procedure described in: Edmondson D. G. and S. J. Norris, "In Vitro

/Sonia Bjorum Brower/ Sonia Bjorum Brower

24 JUL 2024

Technical Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by the contributor to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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²Motility was confirmed by examination by darkfield microscopy following the procedure described in: Edmondson, D. G. and S. J. Norris. "In Vitro Cultivation of the Syphilis Spirochete Treponema pallidum." <u>Curr. Protoc.</u> 1 (2021): e44. PubMed: 33599121. Please refer to this reference before starting work with NR-59701.

³Viability was confirmed by propagation in *Sylvilagus floridanus* epithelial cells (Sf1Ep; ATCC® CCL-68™) at 34°C with a microaerophilic atmosphere (1.5% O₂; 5% CO₂; 93.5% N₂).