

Certificate of Analysis for NR-50142

Anaplasma phagocytophilum, Strain Webster

Catalog No. NR-50142

Product Description:

Anaplasma phagocytophilum (A. phagocytophilum), strain Webster was isolated from the blood of an infected human in May 1996 in Wisconsin, USA. NR-50142 lot 70026731 was produced by infecting HL-60 cells (ATCC[®] CCL-240[™]) and incubating in RPMI-1640 medium containing 10% fetal bovine serum (ATCC[®] 30-2020) for 9 days at 37°C with 5% CO₂.

Lot: 70026731 Manufacturing Date: 12JUL2019

TEST	SPECIFICATIONS	RESULTS
Identification by Indirect Fluorescent Antibody (IFA) Assay ¹	Fluorescence observed	Fluorescence observed
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1340 base pairs)	≥ 99% identity with A. phagocytophilum, strain Webster (GenBank: LANS01000001.1)	100% identity with A. phagocytophilum, strain Webster (GenBank: LANS01000001.1) ²
Titer by TCID₅ Assay in HL-60 Cells by IFA³	Report results	$2.81 \times 10^5 \text{TCID}_{50}$ per mL in 8 days at 37°C with 5% CO ₂
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic ⁴	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 5% CO ₂	No growth	No growth
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic ac	id None detected	None detected

¹Using A. phagocytophilum primary antibody EEDP (Fuller Laboratories) and secondary antibody HCG-25 (Fuller Laboratories)

/Heather Couch/

Heather Couch 18 APR 2022

Program Manager or designee, ATCC Federal Solutions

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²Also consistent with *Ehrlichia equi* and "HGE agent;" however, these species were recently recognized as *A. phagocytophilum*. For more information, please see Dumler, J. S., et al. "Reorganization of Genera in the Families *Rickettsiaceae* and *Anaplasmataceae* in the Order Rickettsiales: Unification of Some Species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, Descriptions of Six New Species Combinations and Designation of *Ehrlichia equi* and 'HGE agent' as Subjective Synonyms of *Ehrlichia phagocytophila*." Int. J. Syst. Evol. Microbiol. 51 (2001): 2145-2165. PubMed: 11760958.

³The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ is the dilution of organism that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of the organism preparation.

⁴Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.