

Certificate of Analysis for NR-46443

Ehrlichia chaffeensis, Strain Heartland

Catalog No. NR-46443

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

Product Description: Ehrlichia chaffeensis (E. chaffeensis), strain Heartland was isolated in 1999 from the blood of a human with acute fatal monocytic ehrlichiosis (HME) in Nebraska, USA.

Lot¹: 70010787 Manufacturing Date: 20NOV2018

TEST	SPECIFICATIONS	RESULTS
Identification by Indirect Fluorescent Antibody (IFA) Assay ²	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (~860 nucleotides)	≥ 98% identity with <i>E. chaffeensis</i> , strain Heartland (GenBank: CP007473.1)	100% identity with <i>E. chaffeensis</i> , strain Heartland (GenBank: CP007473.1)
Titer by TCID ₅₀ Assay ^{3,4} in DH82 Cells ⁵ by IFA ²	Report results	8.9 x 10 ⁵ TCID ₅₀ per mL
Amplification of <i>E. chaffeensis</i> Sequence by PCR	~ 1000 base pair amplicon	~ 1000 base pair amplicon
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Brucella agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic 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) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

¹Grown in Dulbecco's Modified Eagle's Medium containing 4 mM L-glutamine, 4500 mg per L glucose, 1 mM sodium pyruvate and 1500 mg per L sodium bicarbonate (ATCC[®] 30-2002) supplemented with 5% fetal bovine serum (ATCC[®] 30-2020) for 7 days at 37°C with 5% CO₂.

/Heather Couch/

Heather Couch 07 MAR 2019

Program Manager or designee, ATCC Federal Solutions

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²Using *Ehrlichia chaffeensis* IFA IgG reagent kit (Fuller Laboratories ECHG-120)

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.

⁴Assay plates were incubated 14 days at 37°C with 5% CO₂.

⁵Canis familiaris macrophage-monocyte cells (DH-82; ATCC[®] CRL-10389™)

⁶Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798