Certificate of Analysis for NR-48767

Cryptococcus neoformans, Strain H99O

Catalog No. NR-48767

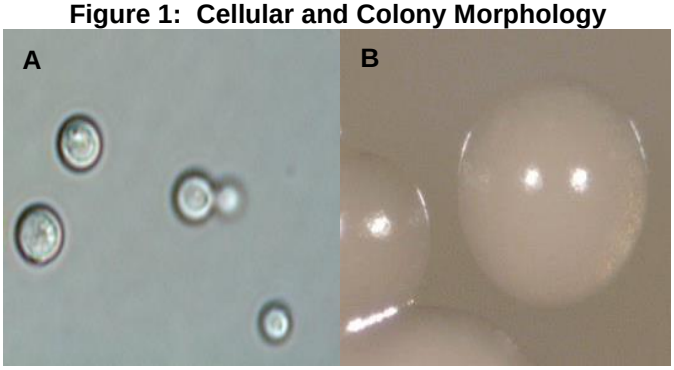
Product Description: *Cryptococcus neoformans (C. neoformans),* strain H99 was isolated from the cerebrospinal fluid of a human male who had been treated for Hodgkin's disease in North Carolina, USA on February 14, 1978. After an unknown number of passages, strain H99 was reported to lose virulence and was subsequently passaged through the rabbit model of infection to increase its virulence. It was renamed as H99O and frozen in 1994.

Lot[1]: 63383701 **Manufacturing Date: 07APR2015**

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology[2]	Report results	Circular yeast form cells, usually single (Figure 1A)
Colony morphology[2]	Report results	Circular, convex, mucoid, shiny and smooth; entire margin (Figure 1B)
CGB agar characterization[3]		
NR-48767	Yellow (no color change)	Yellow (no color change)
Positive control (*C. neoformans;* ATCC® 32045™)	Yellow (no color change)	Yellow (no color change)
Negative control (*C. gattii*; ATCC® MYA-4563™)	Blue	Blue
Genotypic Analysis		
Sequencing of partial 18S ribosomal RNA (rRNA) gene, internal transcribed spacer (ITS) 1, 5.8S rRNA gene, ITS 2, partial 26S rRNA (~ 910 base pairs)	≥ 99% sequence identity to *C. neoformans,* strain H99 (GenBank: CP003821)	99.8% sequence identity to *C. neoformans,* strain H99 (GenBank: CP003821)
Sequencing of 26S rRNA gene (~ 580 base pairs)	≥ 99% sequence identity to *C. neoformans,* strain H99 (GenBank: CP003821)	99.9% sequence identity to *C. neoformans,* strain H99 (GenBank: CP003821)
Purity[4]		
Nutrient broth with 0.1% Yeast Extract at 25°C	No bacterial growth	No bacterial growth
Nutrient broth with 0.1% Yeast Extract at 37°C	No bacterial growth	No bacterial growth
Viability (post-freeze)[2]	Growth	Growth

[1]NR-48767 was produced by inoculation of the deposited material onto Yeast Mold slants and grown 4 days at 30°C in an aerobic atmosphere. Cells were harvested from the slants with 20% glycerol to produce this lot.
[2]3 days at 25°C in an aerobic atmosphere on Modified Sabouraud Dextrose medium
[3]2 days at 35°C in an aerobic atmosphere. CGB medium differentiates *C. gattii* from *C. neoformans* based on the ability of *C. gatti* isolates to grow in the presence of L-canavanine and to assimilate glycine as a sole carbon source, resulting in a blue color. *C. neoformans* isolates will remain yellow. [McTaggart, L., et al. "Rapid Identification of *Cryptococcus neoformans* var. *grubii*, *C. neoformans* var. *neoformans*, and *C. gattii* by Use of Rapid Biochemical Tests, Differential Media, and DNA Sequencing." J. Clin. Microbiol. 2011 (49): 2522-2527. PubMed: 21593254.]
[4]Clarity of broth was determined by visual inspection after 6 days in an aerobic atmosphere.

Figure 1: Cellular and Colony Morphology



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Date: 20 JUL 2016 **Signature:** 

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