

Certificate of Analysis for NR-1400

Bacillus anthracis, Strain Sterne 34F2 (LLNL A0517)

Catalog No. NR-1400

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

Product Description:

Bacillus anthracis (*B. anthracis*) is an aerobic, Gram-positive, spore-forming, rod-shaped bacillus that causes the acute infectious disease anthrax. NR-1400 is a mixture of two colony types. Neither colony type contains the pXO2 plasmid. Only colony type 1 contains the pXO1 plasmid.

Lot: 5352322 Manufacturing Date: 11MAY2006

TEST	SPECIFICATIONS	RESULTS	RESULTS
		Colony Type 1	Colony Type 2
Phenotypic Analysis			
Cellular morphology	Gram-positive rod	Gram-positive rod	Gram-positive rod
Colony morphology			
Tryptic Soy Agar, 5% sheep blood ²	Report results	Circular, entire, ground- glass, grey, sporulation bumps (Figure 1A)	Circular with irregular edges, convex, grey, no sporulation bumps (Figure 1B)
PLET Agar ²	Report results	Circular, entire, ground- glass, white	Circular, entire ground glass, beige
Sporulation	Positive	Positive	Positive
Motility (wet mount)	Non-motile	Non-motile ³	Non-motile ³
β-hemolysis	Non-hemolytic	Non-hemolytic	Non-hemolytic
Capsule (India ink staining)	Report results	Negative	Negative
Tenacious	Positive	Positive	Positive
Analytical profile index (API® 50 CHB)	Consistent with B. anthracis	Consistent with B. anthracis	Consistent with B. anthracis
FAME analysis	Report results	Consistent with B. anthracis	No match
Genotypic Analysis Sequencing of 16S ribosomal RNA gene (~ 1300 base pairs)	Consistent with Bacillus cereus group ⁴	Consistent with Bacillus cereus group ⁴	Consistent with Bacillus cereus group ⁴
PCR Assay of Extracted DNA			
16S ribosomal RNA gene	~ 1500 base pair amplicon	~ 1500 base pair amplicon	~ 1500 base pair amplicon
Presence of virulence plasmids			
pXO1 (<i>aat</i>)	Report results	~ 120 base pair amplicon	No amplicons
pXO2 (at, capA, capB, capC)	No amplicons	No amplicons	No amplicons
Viability (post vialing) ²	Growth	Growth	Growth

¹B. anthracis, strain Sterne 34F2 (Colorado Serum Company vaccine strain) was deposited by Lawrence Livermore National Laboratory. NR- 1400 was prepared by broth/agar culture of the deposited material.

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²24 hours at 37°C and 5% CO₂.

³Performed on NR-1400 (mix of both colony types)

⁴Bacillus cereus group species (*B. cereus, B. thuringiensis, B. mycoides*, and *B. anthracis*) cannot be classified based on 16S sequence (Spencer, R. C. "Bacillus anthracis." J. Clin. Pathol. 56 (2003): 182–187. PubMed: 12610093).



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Figure 1: Colony Morphology





Figure 1. *Bacillus anthracis*, strain Sterne 34F2. Colony type 1 on Tryptic Soy Agar with 5% sheep blood is shown in (A) and colony type 2 is shown in (B).

/Sonia Bjorum Brower/ Sonia Bjorum Brower

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Technical Manager or designee, ATCC Federal Solutions

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