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[1]: 5352322 **Manufacturing Date: 11MAY2006**

TEST	SPECIFICATIONS	RESULTS	
Phenotypic Analysis		**Colony type 1**	**Colony type 2**
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	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 bp amplicon	~ 1500 bp amplicon	~ 1500 bp amplicon
Presence of virulence plasmids			
pXO1 (*aat*)	Report results	~ 120 bp amplicon	No amplicon
pXO2 (*at*, *capA, capB, capC*)	No amplicons	No amplicons	No amplicons
Viability (post-vialing)[2]	Growth	Growth	

[1] *B. 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.
[2] 24 hours at 37°C and 5% CO_2.
[3] Performed on NR-1400 (mix of both colony types)
[4] *Bacillus 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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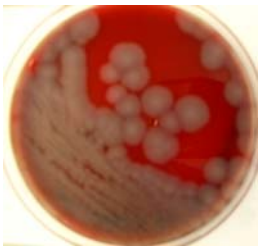
A)



B)

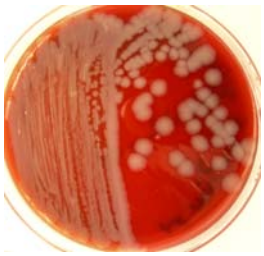


Figure 1. *Bacillus anthracis*, strain Sterne 34F2. Colony type 1 on Tryptic Soy Agar with 5% sheep blood is shown in (A) and colony type 2 is shown in (B).

Date: 10 MAR 2009 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected 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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