Certificate of Analysis for NR-9545

Diarrheagenic *Escherichia coli* Organism Panel

Catalog No. NR-9545

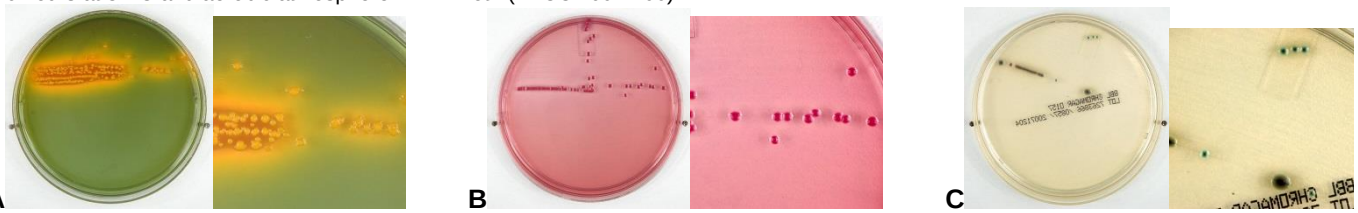
Product Description: NR-9545 consists of five organisms representing different diarrheagenic *Escherichia coli* (*E. coli*) pathotypes. The indicated pathotypes have been confirmed by PCR amplification of marker sequences from extracted nucleic acid.

Lot: 58047883

NR-4, Lot 3561329 (ETEC; Manufactured 31MAR2004)[1]

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology		
Tryptic soy agar	Circular, low convex, undulate, translucent	Circular, low convex, undulate, translucent
Hektoen enteric agar	Bright yellow-orange	Bright yellow-orange (see Figure 1A)
Sorbitol-MacConkey agar	Report results	Red (see Figure 1B)
CHROMagar™ O157	Report results	Blue (see Figure 1C)
Analytical profile index (API® 20 E)	Consistent with *Escherichia coli*	Consistent with *Escherichia coli*
Genotypic Analysis of Extracted Nucleic Acid		
Sequencing of 16S rRNA gene (~ 580 bp)	Consistent with *Escherichia coli*	Consistent with *Escherichia coli*
PCR amplification of plasmid markers		
elt (pJY11)	Report results	Positive
esth and/or *estp* (pCS1)	Report results	Positive
EAF (pEAF)	Negative	Negative
bfpA (pEAF)	Negative	Negative
invE (pINV)	Negative	Negative
CVD432 (pAA)	Negative	Negative
aggR (pAA)	Negative	Negative
PCR amplification of chromosomal markers		
astA	Report results	Positive
eaeA	Negative	Negative
stx1	Negative	Negative
sx2	Negative	Negative
Viability (post-freeze)[3]	Growth	Growth

[1]NR-4 was produced by inoculation of ATCC® 35401™ into Trypticase Soy Broth (BD 211768).
[2]Also consistent with *Shigella* species.
[3]16 hours at 37°C and aerobic atmosphere in LB Broth (ATCC® 60-2100).



A B C

Figure 1

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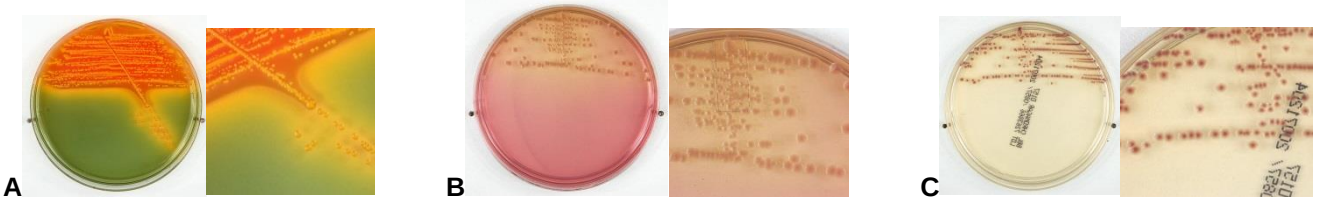
Certificate of Analysis for NR-9545

NR-11, Lot 3560110 (EHEC; Manufactured 05MAR2004)[1]

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology		
Tryptic soy agar	Circular, low convex, entire or undulate, translucent	Circular, low convex, entire or undulate, translucent
Hektoen enteric agar	Bright yellow-orange	Bright yellow-orange (see Figure 2A)
Sorbitol-MacConkey agar	Report results	Colorless (see Figure 2B)
CHROMagar™ O157	Report results	Mauve (see Figure 2C)
Analytical profile index (API® 20 E)	Consistent with *Escherichia coli*	Consistent with *Escherichia coli*
Genotypic Analysis of Extracted Nucleic Acid		
Sequencing of 16S rRNA gene (~ 560 bp)	Consistent with *Escherichia coli*	Consistent with *Escherichia coli*
PCR amplification of plasmid markers		
hylA (pO157)	Positive	Positive
elt (pJY11)	Negative	Negative
esth, estp (pCS1)	Negative	Negative
EAF (pEAF)	Negative	Negative
bfpA (pEAF)	Negative	Negative
invE (pINV)	Negative	Negative
CVD432 (pAA)	Negative	Negative
aggR (pAA)	Negative	Negative
PCR amplification of chromosomal markers		
eaeA	Positive	Positive
stx1	Report results	Positive
sx2	Report results	Positive
astA	Report results	Negative
Viability (post-freeze)[3]	Growth	Growth

[1]NR-11 was produced by inoculation of ATCC® 700927™ into Trypticase Soy Broth (BD 211768).
[2]Also consistent with *Shigella* species.
[3]16 hours at 37°C and aerobic atmosphere in LB Broth (ATCC® 60-2100).



A **B** **C**

Figure 2

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Certificate of Analysis for NR-9545

NR-99, Lot 3663825 (EPEC; Manufactured 01APR2004)[1]

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology		
Tryptic soy agar	Circular, low convex, entire, transparent	Circular, low convex, entire, transparent
Hektoen enteric agar	Bright yellow-orange	Bright yellow-orange (see Figure 3A)
Sorbitol-MacConkey agar	Report results	Colorless (see Figure 3B)
CHROMagar™ O157	Report results	Mauve/blue (see Figure 3C)
Analytical profile index (API® 20 E)	Consistent with *Escherichia coli*	Consistent with *Escherichia coli*
Genotypic Analysis of Extracted Nucleic Acid		
Sequencing of 16S rRNA gene (~ 650 bp)	Consistent with *Escherichia coli*	Consistent with *Escherichia coli*
PCR amplification of plasmid markers		
EAF (pEAF)	Report results	Positive
bfpA (pEAF)	Report results	Positive
elt (pJY11)	Negative	Negative
esth and/or *estp* (pCS1)	Negative	Negative
invE (pINV)	Negative	Negative
CVD432 (pAA)	Negative	Negative
aggR (pAA)	Negative	Negative
PCR amplification of chromosomal markers		
eaeA	Report results	Positive
stx1	Negative	Negative
sx2	Negative	Negative
astA	Report results	Negative
Viability (post-freeze)[3]	Growth	Growth

[1]NR-99 was produced by inoculation of ATCC® 12807™ into Trypticase Soy Broth (BD 211768).
[2]Also consistent with *Shigella* species.
[3]16 hours at 37°C and aerobic atmosphere in LB Broth (ATCC® 60-2100).

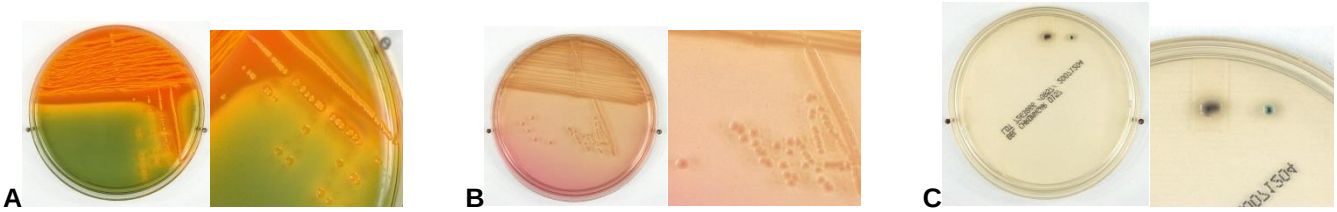

Figure 3

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Certificate of Analysis for NR-9545

NR-100, Lot 3670406 (EIEC; Manufactured 07APR2004)[1]

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology		
Tryptic soy agar	Circular, low convex, entire, smooth, transparent	Circular, low convex, entire, smooth, transparent
Hektoen enteric agar	Bright yellow-orange	Bright yellow-orange (see Figure 4A)
Sorbitol-MacConkey agar	Report results	Red (see Figure 4B)
CHROMagar™ O157	Report results	Mauve/blue (see Figure 4C)
Analytical profile index (API® 20 E)	Consistent with *Escherichia coli*	Consistent with *Escherichia coli*
Genotypic Analysis of Extracted Nucleic Acid		
Sequencing of 16S rRNA gene (~ 580 bp)	Consistent with *Escherichia coli*	Consistent with *Escherichia coli*
PCR amplification of plasmid markers		
invE (pINV)	Positive	Positive
elt (pJY11)	Negative	Negative
esth and/or *estp* (pCS1)	Negative	Negative
EAF (pEAF)	Negative	Negative
bfpA (pEAF)	Negative	Negative
CVD432 (pAA)	Negative	Negative
aggR (pAA)	Negative	Negative
PCR amplification of chromosomal markers		
eaeA	Negative	Negative
stx1	Negative	Negative
sx2	Negative	Negative
astA	Report results	Negative
Viability (post-freeze)[3]	Growth	Growth

[1]NR-100 was produced by inoculation of ATCC® 43892™ into Trypticase Soy Broth (BD 211768).
[2]Also consistent with *Shigella* species.
[3]16 hours at 37°C and aerobic atmosphere in LB Broth (ATCC® 60-2100).

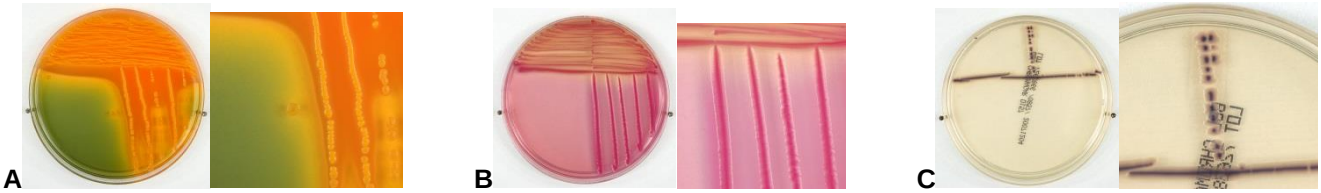

Figure 4

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Certificate of Analysis for NR-9545

NR-102, Lot 3670409 (EAEC; Manufactured 08APR2004)[1]

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology	Gram-negative rods	Gram-negative rods
Colony morphology		
Tryptic soy agar	Circular, low convex, entire, smooth, transparent	Circular, low convex, entire, smooth, transparent
Hektoen enteric agar	Bright yellow-orange	Bright yellow-orange (see Figure 5A)
Sorbitol-MacConkey agar	Report results	Red (see Figure 5B)
CHROMagar™ O157	Report results	Blue (see Figure 5C)
Analytical profile index (API® 20 E)	Consistent with *Escherichia coli*	Consistent with *Escherichia coli*
Genotypic Analysis of Extracted Nucleic Acid		
Sequencing of 16S rRNA gene (~ 580 bp)	Consistent with *Escherichia coli*	Consistent with *Escherichia coli*
PCR amplification of plasmid markers		
CVD432 (pAA)	Report results	Positive
aggR (pAA)	Report results	Positive
elt (pJY11)	Report results	Negative
esth and/or *estp* (pCS1)	Report results	Negative
EAF (pEAF)	Negative	Negative
bfpA (pEAF)	Negative	Negative
invE (pINV)	Negative	Negative
PCR amplification of chromosomal markers		
eaeA	Negative	Negative
stx1	Negative	Negative
sx2	Negative	Negative
astA	Report results	Negative
Viability (post-freeze)[3]	Growth	Growth

[1]NR-102 was produced by inoculation of ATCC® 23501™ into Trypticase Soy Broth (BD 211768).
[2]Also consistent with *Shigella* species.
[3]16 hours at 37°C and aerobic atmosphere in LB Broth (ATCC® 60-2100).

Figure 5

Date: 25 JAN 2010

Signature: Signature on File

Title: Technical Manager, BEI Authentication

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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