



NeoSeek™ STEC – AOAC 081901

SCOPE

This method is applicable for detection and identification of genotypes of *E. coli* O157:H7 and top six non-O157 Shiga toxin-producing *E. coli* (O26, O45, O103, O111, O121 and O145) in raw beef trim and enrichment samples.

Note – if a screen positive sample is to be confirmed by the NeoSeek STEC method, enrichment aliquots of screen positive sample should be shipped to a NeoSeek STEC confirmatory laboratory using the STEC shipping kit or equivalent.

PRINCIPLES

NeoSeek™ utilises multiplex PCR method mass spectrometry for detection and identification of genotypes of *E. coli* O157:H7 and top six non-O157 STEC. Extracted DNA samples undergo PCR amplification followed by primer extension reactions to generate allele-specific DNA products of different masses. Chip-based mass spectrometry analysis using the Agena Bioscience MassARRAY® platform detects more than 80 targets related to virulence and serotype. The resulting molecular profile allows identification of STEC strains. (Targeted genes include *stx*, *eae*, *nle*, and *espA*, among others, including genes for somatic (O) and flagellar (H) antigens).

The detection of STEC can be broken down into the following steps:

- **Sample Enrichment**
Raw meat sample (i.e. 325 ± 32.5 g) is placed into a sterile bag with mesh filter and 975 ± 19.5 mL modified TSB¹ is added. The sample is stomached or hand massaged and incubated at 42 ± 1°C for 18–20 h. A positive control must be included. It is also recommended that a negative control and a blank are also run with each batch of samples.
- **Sample Preparation**
DNA extraction must be carried out as per manufacturer’s instructions.
- **NeoSeek Method protocol²**
Manufacturer’s instructions must be followed for all steps involved within the NeoSeek method protocol². Steps include amplification of extracted DNA samples by multiplex PCR assay followed by primer extension reactions to generate allele-specific DNA products of different masses. Amplified DNA products are analysed by chip-based mass spectrometry to provide allele-specific yields of target genes. This molecular profile is then utilised for molecular identification of STEC strains.
- **Interpretation**
A result of “STEC” indicates that the sample is positive for one of the top seven O groups and the molecular profile generated is consistent with one the top seven STEC strains (minimally *stx*+ and *eae*+). A result of “Non” indicates that the sample is positive for one of the seven O groups but the molecular profile is not consistent with one of the top seven STEC strains.

A result of “Not Detected” indicates that the sample is negative for all of the seven O groups.
- **Confirmation**
Samples that test positive can be further confirmed by cultural method (MLG 5C) from the original enrichment broths.

¹ Modified Tryptone Soya Broth (Oxoid # CM0989B or equivalent) 33.0 g; Casaminoacids (casein acid hydrolysate) 10.0 g; Sterile water 1.0 L. Rehydrate by stirring, then autoclave 20 min at 121°C. Final pH 7.4 ± 0.2 at 25°C.

² Hosking et al. NeoSeek™ STEC: A Multiplex Molecular Method for Detection and Identification of Select Shiga Toxin–Producing *Escherichia coli* in Beef. AOAC Performance Tested Method SM 081901. Journal of AOAC International, Vol. 103, No. 2, 2020.

CHECKLIST

Enrichment	Is enrichment step carried out?	_____
	If yes:	
	Is the sample enriched in mTSB?	_____
	Is enrichment carried out at 42 ± 1 °C for 18-20 h?	_____
	Is a positive control run with each batch of samples analysed?	_____
	Are control cultures inoculated into enrichment broth at a level of 10 to 100 cells?	_____
	If no:	
DNA Extraction	Is correct temperature used for heating, i.e.100±4°C?	_____
	Is correct centrifuge speed used, i.e. 12000 x g?	_____
GeneSeek process	Are the manufacturer’s instructions reproduced in the laboratory manual and followed without modification?	_____
	Is a PCR internal control run?	_____
	Are enrichment control, negative and seven STEC DNA controls run?	_____
	Is PCR grade water used in primer post-extension clean up?	_____
	What volume of PCR product is used for mass spectrometry bio array?	_____
	Are data QC checked and adjusted as appropriate?	_____
	Are technicians familiar with and trained in the operation of GeneSeek equipment?	_____
	Are NeoSeek STEC kits stored as per instructions?	_____
Confirmation	Is cultural confirmation carried out?	_____