# NeoSeekTM 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

NeoSeekTM 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 *esp*A, 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 TSB[[1]](#footnote-1) 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 protocol[[2]](#footnote-2)

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 a DAFF approved cultural confirmatory method from the original enrichment broths.

**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:** |  |
|  | Is enrichment broth received within required conditions (i.e. <7°C, correct shipping kit) and recorded? |  |
| 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? |  |

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. [↑](#footnote-ref-1)
2. Hosking et al. NeoSeekTM 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. [↑](#footnote-ref-2)