# Pall GeneDisc® Plate STEC and STEC Top 6 methods for detection of O157 and top 6 non-O157 Shiga-toxin producing *E. coli* in raw ground beef and beef trim

## SCOPE

The GeneDisc® Plate methods are applicable for detection of Shiga-toxin producing *Escherichia coli* (STEC)serogroups O157, O26, O45, O103, O111, O121 and O145 in raw ground beef and beef trim.

## PRINCIPLES

The Pall GeneDisc® technology uses a real-time PCR technique that combines a dedicated consumable, the GeneDisc® Plate, with a proprietary PCR platform, the Gene Disc® Cycler. Initially the organisms are allowed to grow in buffered peptone water (BPW) broth followed by DNA extraction using Extraction Pack Food 1 protocol. DNA samples then undergo PCR analysis for the presence of the *stx* and *eae* genes and genes specific to O157 using GeneDisc® Plate STEC Detection Kit. Samples positive for *eae* and *stx* genes undergo further PCR analysis for detection of genes specific to O111, O26, O103, O121, O45 or O145 serogroups using GeneDisc® Plate STEC Top 6 Detection Kit. **Enrichment**

A sample weighing 375 ±37.5 g is diluted in 1.5 L pre-warmed (41.5 ± 1°C) BPW broth, homogenized by hand massaging for 2 minutes, and incubated at 41.5 ± 1°C for 10-20 hours. The temperature of both the broth and sample must be at 41.5 ± 1°C for a minimum of 10 h. A positive culture control must be run through all enrichment and testing procedures daily or when testing is carried out.

### GeneDisc® PCR assay for screening STEC

Bacterial DNA is extracted from the enriched samples using the Extraction Pack Food1 protocol and are analysed using GeneDisc® Plate STEC for detection of *stx* and *eae* genes and genes specific to O157. Samples positive for *stx* and *eae* genes are further analysed using GeneDisc® Plate Top 6 Detection Kits for the presence of genes specific to O111, O26, O103, O121, O45 or O145 serogroups. DNA extraction and PCR must be performed as per the manufacturer’s recommended protocols.

### Confirmation

Samples that test PCR negative are reported as negative. Confirmation must be carried out as per USDA-FSIS or equivalent methods for sample enrichments that test GeneDisc Plate STEC or STEC Top 6 PCR positive or have an invalid result. Or, the laboratory may review the cause of the invalid result and based on the findings re-analyse the sample by:

* Repeating the PCR analysis
* Preparing new DNA samples and repeating the analysis or
* Screen testing with another DAFF approved method

Confirmation must be carried out at a DAFF approved laboratory using a DAFF approved method(s).

## CHECKLIST

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| **Enrichment** | Is BPW broth pre-warmed at 41.5 ± 1°C before use? |  |
|  | Is enrichment carried out 41.5 ± 1°C and is the enrichment broth and sample at 41.5 ± 1°C for a minimum of for 10 h? |  |
|  | Is the correct amount of enrichment broth used, i.e. 1.5L? |  |
|  | Is a positive control culture run with each batch of samples? |  |
|  | Is the control culture inoculated into the primary enrichment broth at a level of 10 to 100 cells? |  |
|  | Are the enriched samples also analysed for specific STEC groups using GeneDisc® Plate STEC Top 6 detection kits? |  |
| **GeneDisc® Assay** | Are manufacturer’s instructions available for reference? |  |
|  | Are internal controls run with each batch of samples? |  |
|  | Are technicians familiar with and trained in the operation of the GeneDisc® Technology? |  |
|  | Is the shelf-life of media and kits controlled? |  |
| **Confirmation** | Is isolation carried out at a DAFF approved laboratory using a DAFF approved method(s)? |  |