

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 0157, 026, 045, 0103, 0111, 0121 and 0145 in raw ground beef and beef trim.

PRINCIPLES

The Pall GeneDise® 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 0157 using GeneDisc® Plate STEC Detection Kit. Samples positive for *eae* and *stx* genes undergo further PCR analysis for detection of genes specific to 0111, 026, 0103, 0121, 045 or 0145 serogroups using GeneDise® Plate STEC Top 6 Detection Kit. All samples identified as positive using this test must be confirmed at a department approved laboratory using department approved method(s).

Enrichment

A sample weighing 375 g is diluted in 1.5 L pre-warmed (41.5 \pm 1°C) BPW broth, homogenized by hand massaging for 2 minutes, and incubated at 41.5 \pm 1°C for 10-20 hours. The temperature of both the broth and sample must be at 41.5 \pm 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 0157. Samples positive for *stx* and *eae* genes are further analysed using GeneDisc[®] Plate Top 6 Detection Kits for the presence of genes specific to 0111, 026, 0103, 0121, 045 or 0145 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 department approved method

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

CHECKLIST		
Enrichment	Is BPW broth pre-warmed at 41.5 ± 1°C before use?	
	Is enrichment carried out $41.5 \pm 1^{\circ}$ C and is the enrichment broth and sample at $41.5 \pm 1^{\circ}$ C for a minimum of for 10 h?	
	Is the correct amount of enrichment broth used ie 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?	
	Is 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 department approved laboratory using a department approved method(s)?	