Australian Government
Department of Agriculture

Life Technologies RapidFinder™ STEC Detection Workflow for detection of top 7 STEC serogroups in beef products

SCOPE

This method is applicable for detection of top 7 STEC serogroups (O157, O26, O45, O103, O111, O121 and O145) in beef products.

PRINCIPLES

The RapidFinder™ STEC Detection Workflow operates on a two-stage qPCR process: a) the first stage uses the RapidFinder™ STEC Screening Assay to detect the presence of *eae*, stx_1/stx_2 and the presence of *E. coli* O157:H7 and b) the second stage uses the RapidFinder™ STEC Confirmation Assay to confirm the presence of Top 7 STEC serogroups if the screening assay is positive.

Detection of target STEC involves the following steps:

- **Enrichment**
  Samples (375 g) are placed in 1.0 L of pre-warmed (48°C) Trypticase Soy Broth (TSB). Incubation is carried out for 8 h at 42°C. The temperature of the broth and sample must be at 42°C for a minimum of 8 h. A positive control culture (ie *E. coli* O157 *stx* negative control) must be run through all procedures daily or when testing is carried out.

- **PCR Screening**
  DNA samples are extracted using PrepSEQ(R) Nucleic Acid Extraction Kit for STEC Detection on MagMAX™ Express-96 Magnetic Particle Processors following the manufacturer's instructions. Extracted DNA samples are screened for the presence of STEC using the RapidFinder™ STEC Screening Assay as per manufacturer's recommended protocol. Samples that are negative after the initial screen are reported as negative.

- **PCR Confirmation**
  Screened samples identified as positive, must be analysed for top 7 serogroups using the RapidFinder™ STEC Confirmation Assay. PCR positive samples will be considered as potential positives.

- **Confirmation of Positive results**
  Positive samples must be confirmed using a department approved method at a department approved laboratory or the product deemed positive for one of the identified STEC for the purposes of disposition.

  Confirmation must be carried out as per USDA-FSIS or equivalent methods for sample enrichments that test potential positive, indeterminate, or have an invalid result. The laboratory may choose to review indeterminate or invalid results, and based upon their findings, re-analyse the sample by:

  - Repeating the RapidFinder™ STEC Assays using the primary enrichment broth.
  - Screening using another department approved method.

CHECKLIST

Enrichment	Are samples enriched in TSB pre-warmed at 48°C before use?	_____
	Is enrichment carried out 42°C and is the enrichment broth and sample at 42 °C for a minimum of for 8 h?	_____
	Is the correct amount of enrichment broth used (ie 1.0 L TSB)?	_____
	Is a positive control culture run with each batch of samples/daily?	_____
	Are reference cultures inoculated into enrichment media at a level of 10-100 cells/mL?	_____
PCR 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 Applied Biosystems (Life Technologies) automated System including MagMAX™ Express-96 Instrument?	_____
	Is the shelf-life of media and kits controlled?	_____
Confirmation	Is cultural confirmation carried out using an approved method at a department approved laboratory?	_____