# Life Technologies RapidFinderTM 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**TM** STEC Detection Workflow operates on a two-stage qPCR process: a) the first stage uses the RapidFinder**TM** STEC Screening Assay to detect the presence of *eae*, *stx1/stx2* 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 MagMAXTM Express-96 Magnetic Particle Processors following the manufacturer’s instructions. Extracted DNA samples are screened for the presence of STEC using the RapidFinderTM 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 RapidFinderTM 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 using a DAFF approved confirmatory method 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 RapidFinderTM STEC Assays using the primary enrichment broth.
* Screening using another DAFF 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 MagMAXTM Express-96 Instrument? |  |
|  | Is the shelf-life of media and kits controlled? |  |
| **Confirmation** | Is cultural confirmation carried out using a DAFF approved method at a DAFF approved laboratory? |  |