Detection, Isolation and Identification of top seven Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat and Meat Products - MLG 5C Appendix 3, 4 and 5 (screening only)

SCOPE

Applicable for screening of top six Shiga-toxin producing *E. coli* (STEC) (*E. coli* O26, O45, O103, O111, O121 and O145) in meat products using MLG 5C Appendix 3, and E. coli O157:H7 using MLG 5C Appendix 5.

PRINCIPLES

MLG 5C Appendix 3 and 5 use multiplex Real-Time PCR detection assays utilizing the ABI 7500 FAST[1] platform. The assay detects the presence of the Shiga toxin (*stx1/2*) and intimin (*eae*) genes and genes specific to top seven STEC serogroups.

Note: Primer and Probe Sequences and reagent concentrations must be used as per MLG 5C Appendix 4 (non-O157 STEC) and Appendix 5 (O157:H7).

- **Enrichment**
  Samples (325 ± 32.5 g) are diluted in 975 ± 19.5 mL mTSB. If meat pieces are overweight, prepare a second sub-sample that must be ≥ 63 and ≤ 357.5 g at 1:4 dilution. Samples and diluent are stomached and incubated static at 42 ± 1 °C for 15-24 h. A positive control (*E. coli* O157:H7 *stx*+, *eae*+) and a blank must be included with each batch of samples.

  Note: DAFF export sample weight collected is 375 ± 37.5 g, hence, when using this method, a sub-sample needs to be prepared and analysed.

- **Screening procedure using Real-time PCR for stx/*eae* and O-group**
  DNA Extraction
  DNA extraction is to be conducted from overnight enrichment as per MLG 5C Appendix 3.

  Real-time PCR Procedure
  Samples are screened for the presence of *stx* and *eae* using Real-time PCR assays (follow MLG 5C Appendix 3). Samples negative for *stx* and/or *eae* targets are considered negative for top 7 STEC. Samples that test positive will be further analysed by three additional Real-time PCR Assays to determine if a top six serogroup (O26, O45, O103, O111, O121 or O145) is present. Real-time PCR as per MLG 5C Appendix 5 is used to detect the fliCH7 gene of *E. coli* (including O157:H7). Samples negative for these serogroups are considered negative for top seven STEC.

- **Confirmation**
  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.

[1] The Real-time PCR assay described in this method has been optimised and validated specifically for the ABI 7500 FAST. Use of other Real-time PCR platforms may require optimisation with other probe quencher and reporter dyes.

CHECKLIST

Enrichment	Is the sample enriched in mTSB?	_____
	Is enrichment carried out at 42 ± 1 °C for 15-24 h?	_____
	Is the correct amount of enrichment broth used (i.e. 975 ±19.5 mL of mTSB for 325 ± 32.5 g sample)?	
	Is a sub-sample processed and enriched at 1:4 dilution (i.e. one portion of meat in three portion of broth)?	
	Are a positive control and a blank run with each batch of samples analysed?	_____
	Are control cultures inoculated into enrichment broth at a level of 10 to 100 cells?	_____
Screening	Is screening for *stx* and *eae* undertaken using Real-time PCR as specified in MLG 5C Appendix 3 and 5?	_____
	Is analysis for serogroup specific genes carried out using Real-time PCR as per MLG 5C Appendix 3 & 5?	_____
	Is a cocktail of top seven STEC cultures run with each PCR?	_____
Confirmation	Is confirmation carried out using an approved method at a department approved laboratory?	_____