Australian Government
Department of Agriculture

Detection and Isolation of non-O157 Shiga-toxin Producing *Escherichia coli* (STEC) from Meat and Meat Products - MLG 5B.05

SCOPE

This method is applicable for detection and isolation of top six non-O157 *Shiga*-toxin Producing *E. coli* (O26, O45, O103, O111, O121 and O145) in meat products.

PRINCIPLES

MLG 5B utilises multiplex Real Time-PCR detection assays followed by cultural isolation. The assay detects the presence of the Shiga-toxin (*stx*) and Intimin (*eae*) genes. Samples positive for these genes then undergo further real-time PCR analysis for specific pathogenic STEC serogroups (specific *wzx* genes). Cultural isolation of the screen-positive sample requires use of immuno-magnetic separation (IMS) using beads coated with antibodies (major six serogroups) followed by plating (with and without acid treatment) on modified Rainbow Agar (mRBA). Colonies are then tested for specific O antigens using latex agglutination and positive colonies purified on Sheep Blood Agar (SBA) and confirmation carried out using PCR and biochemical identification.

The detection of non-O157 STEC can be broken down into the following steps:

- **Enrichment**
  Samples are prepared with a 1:4 ratio of product and enrichment broth (ie 325 ± 32.5 g sample with 975 ± 19.5 mL modified TSB[1]), stomached and incubated static at 42 ± 1 °C for 15-24 h. A positive control must be included, it is also recommended that a negative control (*E. coli* ATCC 25922) and a blank are also run with each batch of samples (or daily).

- **Screening using BAX Real-Time PCR**
  Enriched samples are screened for the presence of *stx* and *eae* using BAX Real-Time PCR assays (follow the current BAX System User's guide , the real-time PCR described in MLG 5B Appendices 1 & 3 is an alternative procedure). Samples negative for *stx* and/or *eae* targets are considered negative for non-O157 STEC. Samples that test positive will be further analysed by Panel 1 and Panel 2 PCRs to determine if a top six serogroup (O26, O45, O103, O111, O121 or O145) is present. Samples negative for these six serogroups are considered negative for non-O157 STEC.

- **Immunomagnetic Separation (IMS)**
  Samples positive by the screening test are potential positives. Isolation of non-O157 STEC is carried out using an Immunomagnetic separation procedure (following the FSIS protocol). After IMS, beads with adhering bacteria are diluted 1:10 and 1:100 and plated onto mRBA. A portion of the enrichment broth is also acid treated for one hour at pH 2 to 2.5. The acid treated sample is diluted 1:1 and 1:10 with E-buffer and subcultured onto mRBA. All four plates are incubated at 35 ± 2 °C for 20-24 h.

  Note: if O157 is also being detected CT-SMAC in addition to mRBA must be used for confirmation

- **Isolation**
  Colonies are picked from all plates and tested for agglutination with O antiserum (at this stage the target O group should be known). At least one colony of each morphological type on each plate is tested using latex. A minimum of five latex positive colonies (from each plate, 20 in total) are streaked onto SBA plates and incubated at 35 ± 2 °C for 16-24 h. Colonies on SBA are checked for latex agglutination before continuing with confirmation..

- **Confirmation**
  Latex positive colonies are confirmed by PCR assays for *stx*/*eae* as described in the screening step followed by biochemical identification using VITEK2 or other validated biochemical kits

[1] Modified Tryptone Soya Broth (Oxoid # CM0989B or current) 33.0 g; Casaminoacids (casein acid hydrolysate) 10.0 g; Sterile water 1.0 L. Rehydrate by stirring, then autoclave 20 min at 121°C.. Final pH 7.4 ±0.2 at 25°C.

CHECKLIST

Enrichment	Is the sample enriched in mTSB ?	
	Is enrichment carried out at 42 ± 1 °C for 15-24 h?	
	Is a positive control 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 BAX Real-time PCR?	
	Is analysis for serogroup specific genes carried out using	
	BAX Real-time PCR Panel 1 and Panel 2?	
IMS	Is IMS used following the FSIS protocol (are all serogroups able to be captured)?	
	What volume of immunomagnetic beads is used for IMS process?	
	Are IMS beads also acid treated (pH 2 – 2.5) for one hour?	
Isolation	Are IMS samples diluted according to the FSIS protocol?	
	Are all diluted samples plated onto mRBA (also CT-SMAC if O157 detected) and incubated at 35 ± 2 °C for 20-24 h?	
Confirmation	Are all morphological types from all plates confirmed by latex agglutination?	
	Are latex positive colonies streaked onto SBA and incubated at 35 ± 2 °C for 16-24 h?	
	Are colonies on SBA verified by latex agglutination?	
	Are latex positive colonies confirmed for *stx*/*eae* using BAX Real-time PCR ?	
	Are biochemical tests carried out on positive isolates to confirm *E. coli*?	