Detection, Isolation and Identification of Top Seven Shiga Toxin-Producing Escherichia coli (STEC) from Meat and Meat Products - MLG 5C.03

SCOPE

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

PRINCIPLES

MLG 5C utilises multiplex Real-Time PCR detection assays (iQ-Check STEC VirX & SerO). followed by cultural isolation. The assay detects the presence of the Shiga toxin (*stx1/2*) and intimin (*eae*) genes. Samples positive for these genes then undergo further PCR analysis (iQ-Check STEC SerO) for specific pathogenic STEC serogroups. Cultural isolation of the screen positive sample requires use of immunomagnetic separation (IMS) using beads coated with antibodies (top seven 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 (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 must be included. It is recommended that a negative control (*E. coli* ATCC 25922) and a blank are also run with each batch of samples (or daily).

  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.

- **Rapid Screening PCR for *stx*/*eae* and O-group**
  Samples are screened for the presence of *stx* and *eae* using iQ-Check STEC VirX PCR assays following the Bio-Rad User's guide (the real-time PCR described in MLG 5C Appendices 3, 4 & 5 is an alternative procedure). Samples negative for *stx* and/or *eae* targets are considered negative for top 7 STEC. Samples that test positive will be further analysed by iQ-Check STEC SerO test PCR to determine if a top 7 serogroup (O157, O26, O45, O103, O111, O121 or O145) is present. Samples negative for these serogroups are considered negative for STEC. A positive result for *stx*/*eae* and O group is defined as potential positive.

- **Isolation and Identification**
  Immunomagnetic separation (IMS) and steaking onto mRBA

  Samples positive by the screening test are potential positives. Isolation of top 7 STEC is carried out using an IMS procedure (following the FSIS protocol). IMS beads shall be used for the specific serogroup identified by the serogroup PCR. After IMS, beads with adhering bacteria are diluted 1:10 and 1:100 and plated onto mRBA. A portion of the enrichment broth is 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.

  Examination of mRBA

  O157:H7 colonies typically have black or grey colouration. For all six non-O157, see MLG 5C Appendix 2 for colony characteristics.

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

  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. Samples that have no growth on mRBA or colonies that are agglutination negative are reported as negative for STEC. An optional PCR assay may be performed at this stage to verify the sample is presumptive positive.

- **Confirmation**
  Colonies are to be steaked on SBA for confirmation including a positive control of interest. Perform biochemical identification using VITEK 2 GN card or equivalent to identify *E. coli*. Perform serological agglutination to confirm O antigens. Latex positive colonies are confirmed by the iQ-Check STEC VirX *stx*/*eae* screening assay and STECs are confirmed by iQ-Check STEC SerO Assay.

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 iQ-Check STEC VirX PCR assay?	
	Is analysis for serogroup specific genes carried out using iQ-Check STEC SerO PCR assay?	
	Is a cocktail of top seven STEC cultures run with each PCR?	
IMS	Is IMS used following the FSIS protocol (are all serogroups able to be captured)?	
	What volume of immunomagnetic beads is used for the IMS process?	
	Are IMS beads 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	Is confirmation carried out using an approved method at a department approved laboratory?	
	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 iQ-Check STEC VirX?	
	Are latex positive colonies confirmed for serogroups using serogroup specific PCR iQ-Check STEC SerO?	
	Are biochemical tests carried out on positive isolates to confirm *E. coli*?	