## 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]](#footnote-1)), stomached and incubated static at 42 ± 1℃ 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℃ 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℃ 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.

## CHECKLIST

|  |  |  |
| --- | --- | --- |
| **Enrichment** | Is the sample enriched in mTSB? |  |
|  | Is enrichment carried out at 42 ± 1℃ 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℃ for 20-24 h? |  |
| **Confirmation** | Are all morphological types from all plates confirmed by latex agglutination? |  |

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. [↑](#footnote-ref-1)