Australian Government
Department of Agriculture

Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat and Meat products - MLG 5.09

SCOPE

This method is applicable to raw and ready-to-eat meat and meat products.

PRINCIPLES

The detection of *E. coli* O157:H7 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 mTSB[1]), stomached and incubated static at 42 ± 1°C for 15-24 h.
- **Screening test by BAX System Real-time PCR Assay for *E. coli* O157:H7**
  Perform the rapid screening using 20 µL of enriched samples. Follow the current BAX System User's Guide for preparing reagents, performing PCR test and reading the results.
- **Separation and concentration**
  Samples negative by the screening test can be reported as negative for *E. coli* O157 and can be discarded. Samples positive by the screening test are potential positives. Isolation of *E. coli* O157 is carried out using an Immunomagnetic separation procedure (follow manufacturer's instruction or see MLG 5 for more details). Following concentration, immunomagnetic particles with adhering bacteria are subcultured onto Modified Rainbow Agar (mRBA) and CT-SMAC. 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 and CT-SMAC. All plates are incubated at 35 ± 2°C for 20-24 h.
- **Isolation**
  Typical colonies are tested for agglutination with *E. coli* O157 antiserum. Latex positive colonies are streaked onto sheep blood agar plates and incubated at 35 ± 2°C for 16-24 h.
- **Confirmation**
  E. coli O157 is confirmed by the following tests:
  - Biochemical confirmation using VITEK(R)2
  - O157 and H7 confirmation using E. coli O157:H7 latex test agglutination kit (RIM(R) E. coli O157:H7 Latex Test Kit)
  - Shiga toxin/toxin genes confirmation. Toxin is confirmed by toxin assay using Meridian Premier(R) EHEC Kit. When toxin(s) is/are not demonstrated, detection of gene(s) by PCR is used. Alternatively BAX System Real-time PCR Assay STEC Screening (stx, eae) as per MLG 5.09 may be performed in lieu of the toxin assay.

[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 (including controls for *stx*/*eae*)?	
	Are reference cultures inoculated into primary enrichment broth at a level of 10 to 100 cells?	
Screening	Is the screening test carried out according to BAX Real-time PCR assay for E. coli O157:H7?	
Separation & concentration	For positive screening test samples is *E. coli* O157 separated and concentrated from the enrichment broth using IMS?	
Isolation	Are immunomagnetic beads subcultured onto mRBA agar and CT-SMAC?	
	Are mRBA and CT-SMAC agar plates incubated at 35 ± 2°C for 20-24 h?	
Confirmation	Are typical colonies confirmed using a latex agglutination test?	
	Are all latex positive colonies streaked onto sheep blood agar and incubated at 35 ± 2°C for 16-24 h?	
	Are colonies confirmed by toxin assay or by BAX RT PCR STEC Screening Assay?	