Australian Government
Department of Agriculture and Water Resources

Diarrheagenic *Escherichia coli* – Enrichment and isolation of *E. coli* serotype O157:H7 from Foods – FDA BAM Chapter 4A(K)

SCOPE

This method, updated in June 2016, is applicable to the analysis of food in general and is suitable for the enumeration and isolation of *E. coli* O157 from raw ground beef and trim. The approach recommended here allows the qualitative determination of *E. coli* O157 in raw ground beef and trim.

PRINCIPLES

Enrichment and isolation of *E. coli* O157 can be broken down into the following steps:

- **Enrichment**[1]
  A 25 g sample is diluted in 225 mL of mBPWp and incubated at 37 ± 0.5°C for 5 h. One mL of ACV supplement is then added and incubation continued at 42 ± 1°C static overnight (18-24 h). Positive controls are to be used as detailed in BAM Chapter 4A.

- **Real-time PCR Screening**
  Lab must screen samples using Real-time PCR on enriched samples. The Real-time PCR protocol must be as per BAM Chapter 4A and is performed after immuno-magnetic separation. PCR negative samples are regarded as negative. PCR positive samples require cultural confirmation.

- **Isolation**
  E. coli O157 is isolated by diluting the immuno-magnetic separated sample in Butterfield's phosphate buffer and spread plating 0.05 mL in duplicate onto TC-SMAC plates and one chromogenic agar (Rainbow Agar O157 or R&F E. coli O157:H7 agar). Plates are incubated at 37 ± 1°C for 18-24 h. Suspected colonies are confirmed using latex agglutination (Remel kit). All typical colonies are streaked onto TSAYE plates and incubated at 35°C for 18-24 h

- **Confirmation**
  E. coli O157 is confirmed by indole production, lack of β-glucuronidase activity and serological tests. Presence of Shiga toxin or Shiga toxin genes or genetic confirmation of H7, confirms the presence of *E. coli* O157:H7.

[1] Enrichment with the following modification; must use the IMS option and a sample size of 325 g for ground beef, analysed as five separate 65 g portions, 1:10 dilution

CHECKLIST

Enrichment	Is the sample enriched in mBPWp?	
	Is enrichment at 37 ± 0.5°C initially for 5 h?	
	Is 1 mL ACV supplement added and incubation continued at 42 ±1°C for 18-24 h?	
	Is a positive control run with each batch of samples analysed?	
	Are reference cultures inoculated into primary enrichment broth at a level of 10 to 100 cells?	
Separation	Is *E. coli* O157 separated and concentrated from the enrichment broth using IMS?	
	Is a screen Real-time PCR assay performed on IMS broth samples?	
Isolation	Is IMS sample subcultured in duplicate onto TC-SMAC and onto one chromogenic agar plate?	
	Are plates incubated at 37 ± 1°C for 18-24 h?	
	Are suspect colonies streaked onto TSAYE and incubated at 35°C for 18 to 24 h.	
Confirmation	Is *E. coli* O157 confirmed by:	
	Indole production?	
	β-glucuronidase activity?	
	Agglutination of *E. coli* O157 antiserum?	
	Are tests for toxin or toxin genes carried out?	