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

## SCOPE

This method, updated in July 2020, 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]](#footnote-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.

## 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? |   |

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 [↑](#footnote-ref-1)