# Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157 – AS 5013.26:2020

## SCOPE

This method is applicable to products intended for human consumption or for animal feeding stuffs.

## PRINCIPLES

The detection of *E. coli* O157 necessitates four successive stages:

### Enrichment

A test portion[[1]](#footnote-1) is enriched in nine times the weight of pre-warmed modified tryptone soya broth plus novobiocin (mTSB+N) at 41.5°C ± 1°C for 6 h and subsequently for a further 12 to 18 h.

### Separation and concentration

*E. coli* O157 are separated and concentrated using immunomagnetic beads coated with antibodies to *E. coli* O157 after 6 h[[2]](#footnote-2) and again, if necessary, after a further 12 to 18 h incubation.

### Isolation

Immunomagnetic particles with adhering bacteria are subcultured onto cefixime tellurite sorbitol MacConkey agar (CT-SMAC) and a second selective isolation agar (complementary to CT-SMAC agar) of the laboratories choosing. CT-SMAC is incubated at 37°C for 18 to 24 h. The second agar of choice should be incubated following the manufacturer’s recommended procedures.

### Confirmation[[3]](#footnote-3)

Five typical sorbitol negative colonies from CT-SMAC and five typical *E. coli* O157 colonies on the second isolation agar are streaked onto nutrient agar and incubated at 37°C for 18 to 24 h. *E. coli* O157 is confirmed by indole production and agglutination with *E. coli* O157 antiserum.

## CHECKLIST

|  |  |  |
| --- | --- | --- |
| **Enrichment** | Is the sample enriched in mTSB+N? |   |
|  | Is mTSB+N pre-warmed to 41.5°C prior to use? |   |
|  | Is the correct amount of broth used for the weight of sample analysed i.e. 3,375 mL for a 375 g sample? Is enrichment at 41.5 ± 1°C? |   |
|  | Is enrichment carried out at 41.5 ± 1°C incubated for 6 h and then, if necessary, for a further 12 to 18 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 IMS carried out after 6 h and again, if necessary, after a further 12 to 18 h? |   |
| **Isolation** | Are immunomagnetic beads subcultured onto CT-SMAC and a second selective isolation agar? |   |
|  | Are CT-SMAC plates incubated at 37 °C for 18 to 24 h? |   |
|  | Is the second isolation agar incubated at the recommended time and temperature? |   |
| **Confirmation** | Are suspect colonies streaked onto nutrient agar and incubated at 37°C for 18 to 24 h.  |   |
|  | Is *E. coli* O157 confirmed by:  |   |
|  | Indole production? |   |
|  | Agglutination of *E. coli* O157 antiserum? |   |

1. A larger test portion than that initially validated may be used, if a validation/verification study has shown that there are no adverse effects on the detection of *E. coli* O157. This method has not been validated at lower dilution ratios, i.e. 375g in 1000 mL [↑](#footnote-ref-1)
2. In some cases positive results after 6 h incubation can become negative after a further 18 h incubation. [↑](#footnote-ref-2)
3. Commercially available miniaturized biochemical identification kits that permit the identification of sorbitol-negative and indole-positive *E. coli* and latex agglutination kits for *E. coli* O157 may be used, provided appropriate tests with known positive and negative strains are carried out to confirm performance [↑](#footnote-ref-3)