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] 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] 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]**
  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.

[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.

[2] In some cases positive results after 6 h incubation can become negative after a further 18 h incubation.

[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

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?	