Reveal (20-hour) – AOAC 2000.14

SCOPE

This method is applicable for the detection of *E. coli* O157:H7 in environmental swabs of stainless steel, raw ground beef and raw beef cubes.

PRINCIPLES

Specific antibodies for *E. coli* O157:H7 are bound to a colloidal gold and separately to a solid support. *E. coli* O157:H7 reacts with gold conjugated antibodies forming a complex which flows across a lateral flow membrane where it is bound by antibody immobilised on the membrane. Positive reaction is indicated by a coloured line in the sample test window. A line in the test verification window indicates that the analysis has proceeded correctly

- **Enrichment**
  Place environmental swabs of stainless steel into 225 ml of modified trypticase soya broth plus novobiocin (mTSB+N, FDA BAM). Incubate for 20 h at 35 – 37°C. Cool to room temperature. For raw beef samples add 9 x sample weight of modified *E. coli* broth plus novobiocin (mEC+n). Incubate for 20 h at 35 – 37°C. Cool to room temperature. This method has been validated for use with 375g samples enriched in one litre of broth.

- **Immunoassay**
  Carry out the Reveal assay following the manufacturer's instructions, incubating at room temperature. Ensure that broths are equilibrated to room temperature prior to commencing the assay

- **Confirmation**
  The manufacturer's instructions are not to be followed for confirmation of presumptive positives. Confirmation of *E. coli* O157 is to be carried out from enrichment broths using an Immunomagnetic separation procedure ie ISO 16654:2001, FSIS MLG 5 or FDA BAM Chapter 4A. Confirmation must be undertaken out at a department approved laboratory

CHECKLIST

Enrichment	Environmental swabs	
	Is 225 ml of mTSB+n (or Reveal 20h media) used for enrichment of samples?	
	Is novobiocin added on the day of use?	
	Is enrichment carried out at 35 - 37°C for 20 h?	
	Raw meat samples	
	Is mEC+n (or Reveal 20h media) used for enrichment of samples?	
	Is novobiocin added (mEC+n) on the day of use?	
	What weight of sample and volume of enrichment broth are used?	
	Is enrichment carried out at 35 - 37°C for 20 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?	
Immunoassay	Are the manufacturer's instructions available and are they reproduced in the laboratory manual?	
	Are technicians familiar with positive and negative reactions?	
	Is incubation carried out at room temperature?	
Cultural confirmation	Is *E. coli* O157:H7 confirmed from initial enrichment broth at a department approved laboratory?	
	Is an approved method used for confirmation (ISO 16654:2001, MLG 5, FDA BAM Chapter 4A)?	
	Is IMS concentration included in the confirmation methodology?	