Reveal (8-hour) – AOAC 2000.13

SCOPE

This method is applicable for the detection of *E. coli* O157:H7 in 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**
  Sample is diluted in 9 x its weight of 8 Hour medium prepared on the day of use following the manufacturer's instructions. Media should be prepared using pre-warmed (43°C) sterile deionized water. Mix the sample and incubate for 8 h at 42 – 43°C. This method has been validated for 375g samples enriched in one litre of Reveal 8h medium when incubated for 12-14h.

- **Immunoassay**
  Cultures must be inactivated before proceeding with the immunoassay. Transfer 5 ml of enriched broth to a test tube. Inactivate broth for 10 min at 100°C. Cool inactivated broths to room temperature before proceeding with Reveal assay, store remaining broth, which have not been inactivated, at 2 – 8°C for confirmation of presumptive positive samples. Carry out the Reveal assay following the manufacturer's instructions, incubating at room temperature. Ensure that inactivated 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 the initial enrichment broth 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	Is 8 Hour media prepared on the day of use?	_____
	Is sterile water pre-warmed to 43°C prior to preparation of media?	_____
	What weight of sample and volume of enrichment broth are used?	_____
	Is enrichment carried out at 42 - 43°C for 8 h? (12-14h for 375g samples)	_____
	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?	_____