# 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 enrichment using a DAFF approved confirmatory method. Confirmation must be undertaken at a DAFF 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 DAFF approved laboratory? |  |
|  | Is a DAFF approved method used for confirmation? |  |
|  |  |  |