# 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 using a DAFF approved confirmatory method. Confirmation must be undertaken at a DAFF approved laboratory

## CHECKLIST

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| **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 DAFF approved laboratory? |   |
|  | Is a DAFF approved method used for confirmation? |   |
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