# Assurance GDS for Escherichia coli O157:H7 in Selected Foods - AOAC 2005.04 and Assurance GDS E. coli O157:H7 Tq

## SCOPE

These methods are applicable for detection of *E. coli* O157:H7 in meat and meat products.

## PRINCIPLES

GDS is a DNA based amplification assay. Initially the organisms are allowed to grow in a proprietary modified enrichment medium (mEHEC). Any O157 present are then concentrated by using a concentration device (immunomagnetic separation), followed by amplification of highly conserved DNA sequences in the target organisms with specific primers using an automated gene amplification system. Assurance GDSTD *E. coli* Tq is a modified version of AOAC 2005.04 whereby the polymerase enzyme, instead of being added separately by the analyst, is lyophilized with the other PCR reagents inside the amplification tube at the time of manufacturing.

Detection of *E. coli* O157:H7 involves the follow steps:

### Enrichment

Samples are diluted 1:10 in pre-warmed (42 ± 0.5oC) mEHEC medium. This method is also validated for 375 ±37.5 g composite samples diluted in 1.2 L or 1.5 L of mEHEC medium. Incubation is carried out for 8‑18 h at 42 ± 0.5oC. A positive control culture must be run through all procedures daily or when testing is carried out. The sample and enrichment broth must be at the enrichment temperature for at minimum of 8h.

Note: Analysis of samples for O157 and Top 6 STEC should follow the protocols detailed in the GDA MPX Top 7 STEC methodology.

### Immuno-concentration and PCR Assay

AOAC 2005.04

Enriched samples (1mL) are transferred into a sample well containing 20 µL concentrating reagent agitated at 600 rpm for 5 min. Samples are transferred using the PickPen and washed in 35 µL of resuspension buffer. The washed bead-bacteria complex (20 µL) is transferred into amplification tubes containing 10 µL of polymerase buffer and loaded in an Assurance Rotor-Gene™ (follow the manufacturer’s recommended protocol).

Assurance GDS *E. coli* O157:H7 Tq

The Tq format will involve the following procedural changes:

* the volume of resuspension buffer has changed from 35 µL to 45 µL;
* the addition of 10 µL of polymerase to the Amplification Tubes has been eliminated; and
* the volume of sample transferred from the resuspension plate to the Amplification Tubes has changed from 20 µL to 30 µL.

### Interpretation

Upon completion of the assay the Rotor-Gene program will provide a test result. Each test sample will be identified as positive, indicating that the test sample is positive for *E. coli* O157, negative indicating that the test sample is negative for *E. coli* O157, or “No Amp” indicating that amplification did not occur.

A “No Amp” reading may be due to reagent or test failure or operator error. In this event the test must be repeated using the same enrichment cultures. If the result continues to show “No Amp” the equipment supplier must be contacted for technical services. In this case, the enrichment broth must be analysed using an alternate method or the product deem positive for *E. coli* O157:H7 for disposition purposes

### Confirmation of Positive results

Positive samples must be confirmed using a DAFF approved method at a DAFF approved laboratory or the product deemed positive for *E. coli* O157:H7 for the purposes of disposition.

**CHECKLIST**

|  |  |  |
| --- | --- | --- |
| **Enrichment** | Is the sample enriched in mEHEC medium?  |   |
|  | Is enrichment carried out at 42 ± 0.5°C for 8-18 h? |   |
|  | Is the correct amount of enrichment broth used? |   |
|  | Is a positive control run with each batch of samples/daily? |   |
|  | Are reference cultures inoculated into enrichment media at a level of 10-100 cells/mL? |   |
| **Assay** | Are manufacturer’s instructions available for reference (what method is being used)? |   |
|  | What volume of resuspension buffer is used during sample preparation? |   |
|  | What volume of sample wash is added to Amplification tubes? |   |
|  | Is polymerase prepared and added to Amplification tubes on the day of use? |   |
|  | Are internal controls run with each batch of samples? |   |
|  | Are technicians familiar with and trained in the operation of the GDS Automated System and the Rotor-Gene Program? |   |
|  | Is the shelf-life of media controlled? |   |
| **Confirmation** | Is confirmation carried out from the enrichment culture? |   |
|  | Is confirmation carried out using a DAFF approved method at aa DAFF approved laboratory? |   |