# GENE-UP® *E. coli* O157:H7 2 (ECO 2) – AOAC 2019.03

## SCOPE

This method is applicable for testing of fresh raw ground beef and raw beef trim for *E. coli* O157:H7.

## PRINCIPLES

The GENE-UP® *E. coli* O157:H7 (ECO 2) is a qualitative real-time PCR assay. The GENE-UP Thermocycler detects fluorescence at several wavelengths to allow for multi-target detection in the same reaction vessel.

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

- **Enrichment**
  Sample (375 g) is enriched in 1,125 mL of pre-warmed (to 42 ± 1°C) buffered peptone water (BPW). Sample and enrichment media are placed in a stomacher bag and homogenized using a stomacher. Incubation is carried out at 42 ± 1°C for 10 - 24 h. It is essential that the temperature of the broth and sample is at 41.5 ± 1°C for a minimum of 10 h. A positive and a negative control culture must be run through all procedures daily or when testing is carried out.

- **PCR Assay**
  Sample preparation for bacterial DNA extraction and PCR assays is carried out following the manufacturer's recommended protocol. The GENE-UP® *E. coli* O157:H7 2 kit (REF 423108) must be used in conjunction with the GENE-UP® Lysis kit (REF 414057).

- **Interpretation**
  Upon completion of the assay the program will provide a test result. Each test sample will be identified as positive or negative. If the internal positive control is invalid, the test must be repeated using the same enrichment cultures. If the internal positive control for the re-test sample is invalid, the equipment supplier must be contacted for advice, and the enrichment broth must be analysed using an alternate method or the sample deemed positive.

- **Confirmation of positive results**
  For all positive samples and samples with an invalid positive control result, enriched broth must be confirmed for the presence of *E. coli* O157:H7 at a DAFF approved confirmatory laboratory using a DAFF approved confirmatory method.

## CHECKLIST

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| **Enrichment** | Is the enrichment media pre-warmed to 42 ± 1°C before use? | |
| | Is enrichment carried out at 42 ± 1°C and is the enrichment broth and sample at 42 ± 1°C for a minimum of 10 h? | |
| | Is the correct amount of enrichment broth used? | |
| | Is a positive and a negative control run with each batch of samples/daily? | |
| | Are reference cultures inoculated into enrichment media at a level of 10-100 cells per sample? | |
| **PCR Assay** | Are manufacturer's instructions available for reference? | |
| | Are internal controls run with each batch of samples? | |
| | Are correct kits used for the method? | |
| | Are technicians familiar with and trained in the operation of PCR automated instruments and the associated software? | |
| | Is the shelf-life of media and kits controlled? | |
| **Confirmation** | Is confirmation carried out from the enrichment culture (BPW)? | |
| | Is confirmation carried out using a DAFF approved confirmatory method at a DAFF approved laboratory? | |