



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 $42 \pm 1^\circ\text{C}$) buffered peptone water (BPW). Sample and enrichment media are placed in a stomacher bag and homogenized using a stomacher. Incubation is carried out at $42 \pm 1^\circ\text{C}$ for 10 - 24 h. It is essential that the temperature of the broth and sample is at $41.5 \pm 1^\circ\text{C}$ 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 department approved confirmatory laboratory using a department approved confirmatory method.

CHECKLIST

Enrichment

Is the enrichment media pre-warmed to $42 \pm 1^\circ\text{C}$ before use? _____

Is enrichment carried out at $42 \pm 1^\circ\text{C}$ and is the enrichment broth and sample at $42 \pm 1^\circ\text{C}$ 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 department approved confirmatory method at a department approved laboratory? _____
