# Neogen® Molecular Detection Assay (MDA) 2 - E *coli* O157 (including H7) Method - AOAC 2017.01

## SCOPE

This method is applicable to testing of raw ground beef and beef trim.

## PRINCIPLES

The Neogen® Molecular Detection Assay (MDA) 2 – *E coli* O157 (including H7) method used for the rapid detection of *E. coli* O157:H7 in foods. All samples identified as potentially positive for *E. coli* O157 (including H7) using this method must be confirmed using a department approved confirmatory method.

The detection of *E. coli* O157:H7 involves the following steps:

### Sample enrichment

Meat samples (325 g or 375 g[[1]](#footnote-1)) are enriched in 975 mL or 1,125 mL, respectively, of pre-warmed (41.5 ±1°C) BPW ISO enrichment broth. Samples are homogenised (use of filter bags is recommended) for two minutes and incubated at 41.5 ± 1°C for 10-18 hours. 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 minimum of 10 hours.

### Neogen® system for screening *E. coli* O157

*E. coli* O157:H7 is screened in the sample following the manufacturer’s recommended protocol. Neogen® Molecular Detection Assay 2 - *E. coli* O157 (including H7) method uses isothermal amplification of unique DNA target sequences and bioluminescence to detect the amplified sequences. Positive samples will be considered as potential positive.

### Confirmation

Neogen-positive samples must be confirmed for the presence of *E. coli* O157:H7 at a department approved laboratory. In the event of Neogen-inspect or a Neogen-signal-error, the test must be repeated using the same enrichment culture. If the result continues to show ‘inspect or signal-error’, the enrichment broth must be analysed using an alternative department approved method or must be confirmed at a department approved confirmatory laboratory using a department approved confirmatory method.

## CHECKLIST

|  |  |  |
| --- | --- | --- |
| **Enrichment** | Is the BPW ISO enrichment broth warmed to 41.5 ± 1°C before use? |   |
|  | Is the correct amount of broth used for the weight of sample analysed i.e. 975 mL for a 325 g sample or 1,125 mL for a 375 g sample?  |   |
|  |  |   |
|  | Is a positive control culture run with each batch of samples analysed? |   |
|  | Are control cultures inoculated into the enrichment broth at a level of 10 to 100 cells? |   |
|  |  Is enrichment carried out at 41.5 ± 1°C and is the enrichment broth and sample at 41.5 ± 1°C for a minimum of 10 hours? |   |
| **Screening**  | Are the manufacturer’s instructions reproduced in the laboratory manual and followed without modification? |   |
|  | Are technicians familiar with and trained in the operation of Neogen Molecular Detection System? |   |
|  | Is the shelf-life of media, reagents and kits controlled?Are Neogen MDA Assay kits stored at 2-8°C?Are open kits used by 60 days?Are open kits kept in resealed pouches with desiccant inside and stored at 2-8°C? |   |
| **Confirmation** | Is *E. coli* O157:H7 confirmed at a department approved laboratory using a department approved confirmatory method? |   |

1. DAFF has approved the use of a 375 g meat sample in 1,125 mL diluent based on an independent evaluation of the performance of MDA 2 *E. coli* O157 by Agriculture and Food Laboratory, University of Guelph, 28 June, 2020. [↑](#footnote-ref-1)