



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 g¹) 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.

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.

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? _____
