# Neogen® Molecular Detection Assay (MDA) 2 – *Listeria monocytogenes* Method – AOAC 2016.08

## SCOPE

This method is applicable to:

* Environmental samples and other selected foods.

## PRINCIPLES

The Neogen Molecular Detection Assay (MDA) 2 – *Listeria monocytogens* method is used for the rapid detection of *L. monocytogens* in selected foods and environmental samples. All samples identified as positive for *L. monocytogens* using this test must be confirmed, where confirmation is required, using AS 5013.24.1.

The detection of *L. monocytogens* involves the following steps:

### Sample enrichment

A 1:10 dilution of the sample is made in Demi Fraser (DF) broth (includes FAC) pre-warmed to 20 - 25°C. The sample is homogenized for two minutes (use of filter bags is recommended) and then incubated at 37 ± 1°C for 24 – 30 hours. For environmental samples, swabs are diluted with 10 mL and sponges with 225 mL of DF broth (includes FAC). For raw meat (chicken) a dilution of 1:20 is required (25 g in 475 mL broth). A positive control culture must be run through all procedures daily or when testing is carried out. Sponge samples are palpated for two min while swab samples are vortexed for 30 sec before incubating at 37 ± 1°C for 24 – 30 hours.

### Neogen system for screening *Listeria*

*L. monocytogens* is screened in the sample following the manufacturer’s recommended protocol. Neogen MDA 2 - *Listeria monocytogens* method uses isothermal amplification of unique DNA target sequences and bioluminescence to detect the amplified sequences.

### Confirmation

In all cases of Neogen-positive, Neogen-inspect or a Neogen-signal-error the DF broth should be confirmed using AS 5013.24.1. Confirmation must be carried out at a department approved laboratory.

## CHECKLIST

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| Enrichment | Is the DF broth (includes FAC) allowed to equilibrate to 20 - 25°C before use? |   |
|  | Is the correct amount of broth used for the weight of sample analysed?  |   |
|  | Is a positive control culture run with each batch of samples analysed? |   |
|  | Is the control culture inoculated into the primary enrichment broth at a level of 10 to 100 cells? |   |
|  | Is enrichment carried out at 37 ± 1°C for 24 - 30 h? |   |
| 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 and kits controlled? |   |
| Confirmation | Is *L. monocytogens* confirmed using AS 5013.24.1 at a department approved laboratory? |   |