

The MicroSEQ^(R) Real-Time PCR System for Detection of *Listeria monocytogenes* in food – AOAC 011002

SCOPE

This method is applicable to the analysis of meat and meat products

PRINCIPLES

The MicroSEQ^(R) Real-Time PCR System rapidly amplifies specific DNA fragments unique to *Listeria monocytogenes* followed by signal detection in a single reaction. MicroSEQ^(R) Real-Time PCR Kit for *L. monocytogenes* must be used. All samples identified as potentially positive for *L. monocytogenes* using this test must be confirmed using AS 5013.24.1.

The detection of *L. monocytogenes* is broken down into the following stages:

- **Sample enrichment**

A 25 g portion of sample is diluted in 225 mL of pre-warmed (37°C) Buffered Listeria Enrichment Broth (BLEB), homogenised by stomaching for two minutes and incubated at 37°C for four hours. After four hours, acriflavine (10 mg/L), sodium nalidixate (40 mg/L) and cycloheximide (50 mg/L) are added to the enrichment which is further incubated for 20-24 hours. A positive control culture must be run through the enrichment and initial screening procedure daily or when testing is carried out.

- **Sample preparation and PCR screening**

Sample preparation for bacterial DNA extraction is carried out by using the PrepSEQ^(R) Rapid Spin Sample preparation kit or Automated PrepSEQ nucleic acid extraction kit following the manufacturer's recommended protocol. The extracted DNA sample is run in the Real-Time PCR System.

- **Confirmation**

In the case of a positive, 'warning' and single error result the enrichment sample should be tested using AS 5013.24.1 (starting at the appropriate stage of analysis i.e. selective secondary enrichment). Or, based on the findings of a cause analysis, the laboratory may choose to analyse the 'warning' or signal error result sample by repeating the DNA extraction and PCR analysis.

Confirmation must be carried out at a Department of Agriculture approved laboratory.

CHECKLIST

Pre-enrichment	Is BLEB pre-warmed at 37°C before use?	_____
	Is the correct amount of BLEB used for the weight of the sample analysed?	_____
	Are positive control cultures run with each batch of samples?	_____
	Are control cultures inoculated into the enrichment broth at a level of 10 to 100 cells?	_____
	Is enrichment carried out at 37°C for a total of 24-28 hours?	_____
	Are correct amount of selective agents added to the enrichment at the fourth hour of enrichment?	_____
PCR screening	Are manufacturer's instructions available for reference?	_____
	Are technicians familiar with and trained in the operation of the Applied Biosystems Real-Time PCR System?	_____
	Is the shelf-life of media and kits controlled?	_____
Cultural Confirmation	Is confirmation carried out from the enrichment culture?	_____
	Is isolation carried out at a Department of Agriculture approved laboratory using the Department approved method?	_____