Australian Government
Department of Agriculture and Water Resources

DuPont™ BAX® System Real-Time PCR Assay for *Listeria monocytogenes* - AOAC 121402

SCOPE

This method is applicable to processed meat and environmental samples.

PRINCIPLES

The DuPont™ BAX® System Real-Time PCR Assay rapidly amplifies specific DNA fragments unique to *L. monocytogenes*. All samples identified as potentially positive for *Listeria monocytogenes* using this test must be confirmed using AS 5013.24.1.

The detection of *Listeria monocytogenes* is broken down into following stages:

- **Pre-enrichment**
  A 25 g ± 1 g portion is used for analysis of processed meat. Primary enrichment is carried out in 225 ± 5 mL of pre-warmed (30°C) modified University of Vermont broth (UVM) which is incubated at 30 ± 2°C for
  23 - 26 h. Environmental swab samples are enriched in 225± 5 mL of pre-warmed UVM and incubated at 30 ±2°C for 20 - 26 h.

- **Enrichment[1]**
  Primary enrichment culture (0.1 ± 0.02 mL) is transferred to 10 ± 0.5 mL of pre-warmed (35°C) MOPS-BLEB broth. Secondary enrichment is carried out at 35 ± 2°C for 18-24 h.

- **DuPont™ BAX® system Real-Time PCR for screening**
  Listeria monocytogenes is screened in the secondary enrichment broth following the manufacturer's recommended protocol. The BAX *Listeria monocytogenes* test is an automated method that uses polymerase chain reaction (PCR) technology for the detection of *Listeria monocytogenes* in food and environmental samples. The system identifies and amplifies a specific DNA fragment unique to *Listeria monocytogenes*. A fluorescent dye then binds with double-strand DNA and emits a fluorescent signal in response to light. After amplification, the BAX System begins a detection phase in which the fluorescent signal is measured. The system utilises a BAX System Q7 instrument.

- **Confirmation**
  For BAX-positive or BAX-indeterminate results, or a where BAX-signal-error has occurred, the sample must be confirmed using AS 5013.24.1 (starting at the appropriate stage of analysis i.e. plating out and identification). Confirmation must be carried out at a Department of Agriculture and Water Resources approved laboratory.

[1] A single step enrichment using Actero™ *Listeria* Enrichment Media can be performed. Homogenize 125 g sample with 750 mL pre-warmed (35°C) media. Homogenize sponge with 90 mL of pre-warmed media. Incubate at 35°C for 26 – 28 h.

CHECKLIST

Primary enrichment	Is appropriate volume of media used for the sample being analysed?	_____
	Is media pre-warmed (30°C) before use?	_____
	Is the initial suspension incubated at 30 ± 2°C for 23 – 26 h?	_____
	Is a positive control run with each batch of samples analysed?	_____
	Are reference cultures inoculated into primary enrichment broth at a level of 10 to 100 cells?	_____
Secondary enrichment	Is secondary enrichment in MOPS-BLEB used when UVM is used as pre-enrichment?	_____
	Is secondary enrichment carried out at 35 ± 2°C for 20 - 26 h?	_____
BAX RT PCR screening	Are positive and negative controls run with each batch of samples?	_____
	Are the manufacturer's instructions reproduced in the laboratory manual and followed without modification?	_____
Confirmation	Is *Listeria* confirmed using AS 5013.24.1?	_____
(if applicable)	If an external laboratory is used is it department approved?	_____