BAC*Gene Listeria monocytogenes* – AOAC 061703

SCOPE

This method is applicable to meat products and other foods and environmental samples. This method has been validated on 25 g of meat products.

PRINCIPLES

The BAC*Gene Listeria monocytogens* is a qualitative real-time PCR assay that utilizes unique primer and probe components for highly-specific detection of genes unique to *Listeria monocytogens*. Target DNA fragments when detected are amplified and then identified with a R6G fluorescence-labelled hybridization probe quenched by non-fluorescent Tide Quencher 2 (TQ2).

Detection of *Listeria monocytogenes* involves the follow steps:

- **Enrichment**
  Sample (25 g) is enriched in 225 mL of pre-warmed (37± 1°C) Actero *Listeria* Enrichment media for 21 ± 3 h. Sponge samples can be enriched in 100 mL enrichment media or 10 mL if using small swabs. A positive control culture must be run through all procedures daily or when testing is carried out.

- **PCR Assay**
  Sample preparation for bacterial DNA extraction and PCR assay must be carried out following the manufacturer's recommended protocol.

- **Interpretation**
  Upon completion of the assay the program will provide a test result. Each test sample will be identified as positive, indicating that the test sample is positive for *Listeria monocytogenes* or negative indicating that the test sample is negative for *Listeria monocytogenes*. If the internal positive control is invalid, the test must be repeated using the same enrichment cultures. If the internal positive control for the re-test sample is invalid, the equipment supplier must be contacted for advice, and the enrichment broth must be analysed using an alternate method or the sample deemed positive for *Listeria monocytogenes*.

- **Confirmation of positive results**
  For all positive samples and samples with an invalid positive control result, enrichment broth must be analysed using AS 5013.24.1. Confirmation must be carried out at a Department approved laboratory.

CHECKLIST

Enrichment	Is the enrichment media warmed to 37± 1°C before use?	
	Is enrichment carried out at 37 ± 1°C for 21 ± 3 h?	
	Is the correct amount of enrichment broth used?	
	Is a positive control run with each batch of samples/daily?	
	Are reference cultures inoculated into enrichment media at a level of 10-100 cells per sample?	
PCR Assay	Are manufacturer's instructions available for reference?	
	Is a PCR internal positive control run with each batch of samples?	
	Are technicians familiar with and trained in the operation of the PCR automated instruments and the software?	
	Is the shelf-life of media and kits controlled?	
Confirmation	Is confirmation carried out from the enrichment culture?	
	Is confirmation carried out using AS 5013.24.1 at a Department approved laboratory?	