Australian Government
Department of Agriculture and Water Resources

The MicroSEQ(R) Real-Time PCR System for Detection of *Salmonella* in food – AOAC 031001

SCOPE

This method is applicable to the analysis of meat and meat products (including carcase sponges)

PRINCIPLES

The MicroSEQ® Real-Time PCR System rapidly amplifies specific DNA fragments unique to Salmonella followed by signal detection in a single reaction. MicroSEQ® Real-Time PCR Kit for *Salmonella* must be used. All samples identified as potentially positive for *Salmonella* using this test must be confirmed using AS 5013.10.

The detection of *Salmonella* spp. 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 peptone water (BPW). The sample is homogenised by stomaching for 2 min and incubated at 37 ± 1°C for 16-20 h. 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® 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 presence of *Salmonella* should be confirmed using AS 5013.10 (starting at the appropriate stage of analysis i.e. selective enrichment). Or, based on the findings of a cause analysis, the laboratory may choose to re-analyse the 'warning' or signal error result sample by repeating the DNA extraction and PCR analysis.

  Confirmation must be carried out at a Department approved laboratory.

CHECKLIST

Pre-enrichment	Is BPW pre-warmed at 37°C before use?	_____
	Is the correct amount of BPW 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 ± 1°C for 16-20 hours?	_____
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 BPW enrichment culture?	_____
	Is confirmation carried out at a Department approved laboratory using a Department approved method?	_____