Australian Government
Department of Agriculture and Water Resources

foodproof(R) *Salmonella* Detection Kit, 5'Nuclease and Hybridization Probes – AOAC 120301

SCOPE

This method is applicable to:

- Food and environment samples

PRINCIPLES

The **food**proof(R) *Salmonella* Detection Kit rapidly amplifies specific DNA fragments unique to the target organism by PCR. Initially the organisms are allowed to grow in buffered peptone water (BPW) followed by sub cultivation in brain heart infusion (BHI) broth followed by PCR assays for the presence of target genes. Samples identified as positive (initial reactive) must be confirmed using AS 5013.10.

The detection of *Salmonella* spp. is broken down into:

- **Pre-enrichment (ISO 6579)**
  For meat and meat products a 1:10 dilution of the sample is enriched in pre-warmed BPW at 37 ± 1°C for 18 h ± 2 h. For carcass sponges, BPW is added to the moistened sponge to bring the total volume to 60-100 mL and the sample incubated at 37 ± 1°C for 18 h ± 2 h. A positive control culture must be run through the enrichments and initial screening procedure daily or when testing is carried out.

- **Sub cultivation**
  Culture from the pre-enrichment broth is inoculated into pre-warmed BHI broth (1 mL sample + 9 mL broth) and is incubated at 37°C for 3 hours.

- **Screening**
  DNA samples are extracted with **food**proof(R) ShortPrep I Kit or the **food**proof(R) StarPrep One Kit following the manufacturer's instructions. Extracted DNA samples are screened for the presence of specific gene using the PCR with **food**proof(R) *Salmonella* Detection Kit as per manufacturer's recommended protocol. Samples that are negative after the initial screen are reported as negative.

- **Confirmation of *Salmonella***
  In the case of positive, undecided or a signal error the BHI (brain heart infusion broth) should be tested 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 analyse the indeterminate or signal error result sample by repeating PCR analysis.

  Confirmation must be carried out at a department approved laboratory.

CHECKLIST

Pre-enrichment	Is the buffered peptone water warmed to room temperature (or 37°C)?	
	Is pre-enrichment at 37 ± 1°C for 18 h ±2 h?	
	Is the sample sub-cultivated in BHI broth at 37 ± 1°C for 3 hours?	
	Is the correct amount of BPW and BHI broth used for the weight of sample analysed?	
	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?	
foodproof(R) Screening	Are the manufacturer's instructions available?	
	Are technicians familiar with and trained in the operation of the **food**proof(R) real-time PCR system?	
	Is the shelf-life of media and kits controlled?	
Confirmation	Is confirmation carried out from the enrichment culture?	
	Is isolation carried out at a department approved laboratory using a department approved method?	