Australian Government
Department of Agriculture and Water Resources

DuPont Qualicon BAX(R) System *Salmonella* 2 PCR Assay – AOAC 100201

SCOPE

This method is similar to AOAC 2003.09; the difference is proprietary "hot-start" technology in the *Salmonella* 2 PCR tablets that keeps the reaction enzyme inactive until PCR begins. This minor modification greatly reduces the non-specific PCR products to form and improves the specificity of the assay. This method also differs from incubation time and temperature.

This procedure outlined the analysis of:

- Raw meat and poultry

PRINCIPLES

This method uses a commercial PCR screening procedure. 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 four stages:

- **Pre-enrichment in non-selective liquid medium**
  A 1:10 dilution of the sample is enriched in buffered peptone water at 37°C for 16-24h. Buffered peptone water should be warmed to room temperature or to 37°C for large volumes. For carcass sponges, buffered peptone is added to the moistened sponge to bring the total volume to 60-100 mL and the sample incubated at 37°C for 16-24 h. In the case of sponges BPW need not be warmed to room temperature before being used to re-hydrate the sponge, for all subsequent additions BPW should be warmed to room temperature.

- **BAX system for screening *Salmonella***
  Salmonella is screened in the sample following the manufacturer's recommended protocol[1]. The BAX *Salmonella* test is an automated method that uses polymerase chain reaction (PCR) technology for the detection of *Salmonella* in foods. The system identifies a specific DNA fragment, unique to *Salmonella*. The DNA is combined with DNA polymerase, nucleotides, and primers. The mixture then undergoes a series of timed heating and cooling cycles. Heating denatures the DNA, separating it into single strands. As the mixture cools, the primers recognise and bind to the targeted DNA sequences. The DNA polymerase then uses the nucleotides to extend the primers, thus creating 2 copies of the targeted DNA fragment; repeating the cycle results in an exponential increase in the number of target DNA fragments. 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.

- **Confirmation**
  In all cases of BAX-positive, BAX-indeterminate or a BAX-signal-error the BPW must be tested using AS 5013.10 (starting at the selective enrichment stage of the analysis). Confirmation must be carried out at a department approved laboratory.

[1] BAX(R) System PCR Assay *Salmonella* 2 Part # D14368501

CHECKLIST

Pre-enrichment	Is the buffered peptone water warmed to room temperature (to 37°C for large quantities)?	_____
	Is the correct amount of enrichment 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?	_____
	Is enrichment carried out at 37°C for 16-24 h?	_____
BAX screening	Are the manufacturer's instructions available?	_____
	Is the shelf-life of media and kits controlled?	_____
Confirmation	Are all suspect *Salmonella* sent to a reference Laboratory (see AS 5013.10) to be serotyped?	_____
	BPW should be supplied to off-site laboratories for confirmation following AS 5013.10	_____
	If *Salmonella* confirmation is done in-house refer to AS 5013.10.	_____