BAC*Gene Salmonella* spp. – AOAC 121501

SCOPE

This method is applicable to raw ground beef, other foods and environmental samples. This method has been validated in 375 g of raw ground beef samples.

PRINCIPLES

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

Detection of *Salmonella* involves the follow steps:

- **Enrichment**
  Sample (25 g) is enriched in 225 mL of pre-warmed buffered peptone water. Incubation is carried out at 37 ± 1°C for 16 - 24 h. For carcass sponges, BPW is added to the moistened sponge to bring the total volume to 225 mL. Raw meat of 375 g can be diluted in 1:10 pre-warmed buffered peptone water and incubated at 41.5 ± 1°C for 10 – 18 h. 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 is carried out following the manufacturer's recommended protocol. BAC*Gene Salmonella* spp is a PCR based test for qualitative detection of *Salmonella* spp. in selected foods and environmental samples.

- **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 *Salmonella*, or negative indicating that the test sample is negative for *Salmonella*. 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 *Salmonella*.

- **Confirmation of positive results**
  For all positive samples and samples with an invalid positive control result, BPW must be analysed using AS 5013.10 (starting at the selective enrichment stage of the analysis). Confirmation must be carried out at a Department approved laboratory.

CHECKLIST

Enrichment	Is enrichment carried out at 37 ± 1°C for 16 - 24h?	
	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?	
	Are internal controls run with each batch of samples?	
	Are technicians familiar with and trained in the operation of PCR automated instruments and the associated software?	
	Is the shelf-life of media and kits controlled?	
Confirmation	Is confirmation carried out from the enrichment culture (BPW)?	
	Is confirmation carried out using AS 5013.10 at a Department approved laboratory?	