Australian Government
Department of Agriculture and Water Resources

BioControl Assurance EIA - AOAC 992.11

SCOPE

This method is applicable to all foods when modified for raw meat or heavily contaminated samples.

PRINCIPLES

The detection of *Salmonella* spp. in raw meat samples is broken down into stages as follows:

- **Pre-enrichment in non-selective liquid medium**
  A 1:10 dilution of the sample must be pre-enriched in buffered peptone water supplemented with 4 ml of 0.1% novobiocin solution[1] at 35-37°C for 18 to 26 h. Buffered peptone water should be warmed to room temperature or to 36 °C for large volumes. For carcass sponges, buffered peptone supplemented with 0.1% novobiocin solution is added to the moistened sponge to bring the total volume to 60-100 ml and the sample incubated at 35-37°C for 18-26 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.

- **Selective enrichment**
  Culture from the pre-enrichment broth is inoculated into Selenite Cystine (SC) broth (35-37°C) and tetrathionate (TT) broth (42 ± 0.5°C) and incubated at the indicated temperature for 18-24h.

- **Post enrichment**
  Cultures from selective liquid media are combined and inoculate into M broth and incubate at 42 ± 0.5°C for 6-8 h.

- **Enzyme immunoassay**
  Follow the manufacturer's instructions retaining M broth for confirmation of presumptive positive results.

- **Cultural confirmation**
  Presumptive positives can be confirmed from the retained M broth by streaking onto XLD, HE and BS agar[2]. Confirmation carried out at an 'off-site' laboratory must be from retained BPW enrichment. Typical colonies are confirmed as *Salmonella* using biochemical and serological tests as outlined in AS 5013.10.

[1] Suspend 0.1g of novobiocin sodium salt in 100 ml of purified water. Filter sterilise (0.2 μm). Solution is stable up to 60-days in a dark bottle at 2-8 °C.

[2] Xylose Lysine Deoxycholate (XLD), Hektoen enteric (HE), Bismuth Sulphite (BS)

CHECKLIST

Pre-enrichment	Is the buffered peptone water warmed to room temperature (to 36 ± 1°C for large quantities)?	_____
	Is novobiocin added to BPW?	_____
	Is the correct amount of enrichment broth used for the weight of sample analysed?	_____
	What volume is used for carcase swabs?	_____
	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 pre-enrichment done at 36 ± 1°C for 18-26 h?	_____
Selective-enrichment	Are selective enrichment broths incubated at the appropriate temperature?	_____
	Are both selective broths used on the day of preparation?	_____
Post-enrichment	Is M broth incubated at 42 ± 0.5°C for 6-7 h?	_____
	Is M broth retained for confirmation of presumptive positive samples? How is it stored?	_____
	Are selective enrichments cultures combined for analysis?	_____
Enzyme immunoassay	Are the manufacturer's instructions available?	_____
	Are reagents stored at 2-8°C?	_____
	Is incubation carried out at 35-37°C?	_____
	Are kit reagents or components from other batches used in the analysis of samples?	_____
Cultural confirmation	Is *Salmonella* isolated in-house from M broths?	_____
	Are XLD, HE and BS agars used for confirmation?	_____
(if applicable)	If an external laboratory is used is it department approved?	_____
	BPW should be supplied to off-site laboratories for confirmation following AS 5013.10	_____
	Are *Salmonella* confirmed using AS 5013.10 (with regard to biochemical and serological tests)?	_____