Australian Government
Department of Agriculture and Water Resources

BioControl VIP - AOAC 999.09

SCOPE

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

PRINCIPLES

Detection of *Salmonella* is based on binding specific *Salmonella* antigens in an antigen-antibody-chromogen complex. The complex flows across a lateral flow membrane and is subsequently bound by antibody immobilised on membrane. When *Salmonella* is present in the sample a detection-line will form in the viewing window of the device. An internal control is present to allow the operator to determine if the test has been performed correctly. The detection of *Salmonella* spp. 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 to 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 RV broth and TT broth. Selective enrichment broths are incubated at 42 ± 0.5°C for 16-24 h.

- **Post enrichment**
  Cultures from selective liquid media are combined and inoculated into pre-warmed TSB + DNP+n[2] and incubate at 42 ± 0.5°C for 5-8 h.

- **Enzyme immunoassay**
  Follow the manufacturer's instructions retaining TSB + DNP+n for confirmation of presumptive positive results. Samples are lysed by the addition of extraction reagents followed by autoclaving (121 °C for 10 min).

- **Cultural confirmation**
  Presumptive positives can be confirmed from the retained TSB+n by streaking onto XLD, HE and BS agar[3]. 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). Add 4ml to 225ml of buffered peptone water. Solution is stable up to 60-days in a dark bottle at 2-8 °C.

[2] Tryptone soy broth + 2,4,dinitrophenol (0.1 g per litre) + 0.1% novobiocin (novobiocin added after autoclaving)

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

CHECKLIST

Pre-enrichment	Is BPW + n warmed to room temperature (to 36°C for large quantities) prior to use?	
	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 pre-enrichment done at 36 ± 1°C for 18-26 h?	
	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?	
Selective-enrichment	Are selective enrichment broths incubated at the appropriate temperature?	
	Is TT both used on the day of preparation?	
	Are selective enrichments cultures combined for analysis?	
Post-enrichment	Is novobiocin added to the TSB + DNP on the day of testing?	
	Is TSB broth incubated at 42 ± 0.5°C for 5-8 h?	
	Is TSB broth retained for confirmation of presumptive positive samples? How is it stored?	
Enzyme immunoassay	Are the manufacturer's instructions available?	
	Are cells inactivated by autoclaving prior to running the immunoassay?	
	Are VIP units stored at room temperature in a cool dark place?	
	Are technicians familiar with positive and negative reactions?	
	Is incubation carried out at room temperature?	
Cultural confirmation	Is *Salmonella* isolated in-house from TSB 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)?	