Australian Government
Department of Agriculture and Water Resources

# BioControl Assurance Gold - AOAC 999.08

## 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 novobiocin[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 (0.1 ml in 10 mL) and TT broth (1 mL in 10 mL). Selective enrichment broths are incubated at 42 ± 0.5°C for 5 to 8 h.

- **Post enrichment**
  Cultures from selective liquid media are combined and inoculate into pre-warmed TSB+n[2] and incubate at 42 ± 0.5°C for 16-20 h.

- **Enzyme immunoassay**
  Follow the manufacturer's instructions retaining TSB+n for confirmation of presumptive positive results.

- **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). Solution is stable up to 60-days in a dark bottle at 2-8 °C. Added at the rate of 4mL/225mL of BPW.

[2] Tryptone soy broth + 0.1% novobiocin (novobiocin added after autoclaving)

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

## CHECKLIST

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|---|---|---|
| **Pre-enrichment** | Is the buffered peptone water warmed to room temperature (to 36°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? | |
| | Is preparation of TT completed on the day of use? | |
| **Post-enrichment** | Are selective enrichments cultures combined for analysis? | |
| | Is TSB+n broth incubated at 42 ± 0.5°C for 6-7 h? | |
| | Is TSB+n broth retained for confirmation of presumptive positive samples? | |
| **Enzyme immunoassay** | Are the manufacturer's instructions available? | |
| | Are reagents stored at 2-8°C? | |
| | Is incubation carried out for 30 min at 35-37°C? | |
| **Cultural confirmation** | Is *Salmonella* isolated in-house from TSB+n broth? | |
| | 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)? | |