Australian Government
Department of Agriculture and Water Resources

Isolation and identification of *Salmonella* from Meat and Carcass Sponges - MLG 4.09

SCOPE

This method is applicable to:

- Raw meats, meat products, carcass swabs and other foods

PRINCIPLES

The detection of *Salmonella* spp. is broken down into four stages:

- **Pre-enrichment**
  Sample (25 g) is enriched in pre-warmed (42°C) 75 ± 1.5 mL mTSB at 42 ± 1°C for 15-24 h.

  Where a single raw beef product sample is tested for *Salmonella*, *E. coli* O157 and top six STEC, a 325 ±32.5 g sample is enriched in pre-warmed (42°C) 975 ±19.5 mL of mTSB at 42 ± 1°C for 15-24 h. For carcass sponges, mTSB is added to the moistened sponge to bring the total volume to 60-100 mL and the sample incubated at 42 ± 1°C for 15-24 h.

  For Ready-to-Eat foods, 325 ± 6.5 g sample is enriched in 975 ± 19.5 mL of pre-warmed Buffered Peptone Water at 35 ± 2°C for 18-24 hours.

  A positive control culture must be run through all procedures daily or when testing is carried out.

- **Enrichment in selective liquid medium**
  Culture from the pre-enrichment broth is inoculated into tetrathionate (TT) broth (Hajna) and Modified Rappaport-Vassiliadis medium (mRV broth). All broths are incubated at 42 ± 0.5°C for 22-24 h.

- **Plating out and identification**
  Cultures obtained from the selective enrichment are streaked onto two selective media:

  - Xylose lysine tergitol agar (XLT4 agar) or Double modified lysine iron agar (DMLIA).
  - Brilliant green sulfa agar (BGS; contains 0.1% sodium sulphapyridine).

  All plates are incubated at 35 ± 2°C and examined after18-24 h. Suspect colonies are picked for confirmation. All negative plates are re-incubated for 18-24 h and examined for presumptive positive colonies.

- **Confirmation of *Salmonella***

Colonies of presumptive *Salmonella* are subcultured and confirmed by appropriate biochemical tests. Rapid biochemical identification kits described in AOAC 978.24 can be used. *Salmonella* isolates must be sent to a reference laboratory for serotyping.

CHECKLIST

Pre-enrichment	Is the mTSB warmed to 42°C?	_____
	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?	_____
Selective-enrichment	Is RV broth incubated at 42 ± 0.5°C for 22-24 h?	_____
	Is the TT broth incubated at 42 ± 0.5°C for 22-24 h?	_____
	Is TT broth boiled not autoclaved?	_____
	Is the TT broth prepared on the day of use (or has a validated shelf-life as per the manufacturer)?	_____
Selective plating	Are isolated colonies obtained on selective solid media?	_____
	Is the isolation of H_2S negative strains mentioned in the laboratory's methods manual?	_____
Confirmation	Are pure cultures on nutrient agar used for biochemical tests?	_____
	Are approved rapid bio-chemical test kits used?	_____
	Are biochemical tests used sufficient to identify *Salmonella* spp.?	_____
	Are all suspect *Salmonella* sent to a reference laboratory (see AS 5013.10) to be serotyped?	_____