Microbiology of the food chain: Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Detection of *Salmonella* spp. AS 5013.10-2022

This standard is an adoption with national modification of ISO 6579-1:2017. Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp. and its Amendment No. 1. This standard replaces AS 5013.10.2009.

SCOPE

This method is applicable to:

- products intended for human consumption (including raw meats and carcass swab and rinse samples) and the feeding of animals.
- environmental samples in the area of food production and food handling.

PRINCIPLES

Salmonella Hofit is used as the positive control for this method. The detection of *Salmonella* spp. is broken down into four stages:

- **Pre-enrichment in non-selective liquid medium**
  For meat and meat products a 1:10 dilution of the sample is enriched in buffered peptone water at 36 ± 2°C for 18 h ± 2 h. Buffered peptone water should be warmed to room temperature or to 36 ± 2°C for large volumes (i.e. >225 mL). For carcass sponges, buffered peptone water is added to the moistened sponge to bring the total volume to 60-100 ml and the sample incubated at 36 ± 2°C for 18 h ± 2 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.

- **Enrichment in selective liquid medium**
  Culture from the pre-enrichment broth is inoculated into Rappaport-Vassiliadis medium with soya (RVS broth) and Muller-Kauffmann tetrathionate/novobiocin broth (MKTTn broth, pH 8.0 ± 0.2 at 25°C). The RVS broth is incubated at 41.5 ± 1°C for 24 h ± 3 h and the MKTTn broth at 37 ± 1°C for 24 h ± 3 h.

- **Plating out and identification**
  Cultures obtained from the selective enrichment are streaked onto two selective media:
  - Xylose lysine deoxycholate agar (XLD agar)
  - And, for testing as part of export certification, any other solid selective medium that is complementary to XLD and able to detect H_2S negative serovars of *Salmonella* e.g. Brilliant Green Agar (BGA).

  XLD agar is incubated at 37 ± 1°C and examined after 24 h ± 3 h. The second agar is incubated according to the manufacturer's recommendations. The department does not require the use of duplicate 90 to 100 mm Petri dishes or a single 140 mm Petri dishes. Single 90 to 100 mm Petri dishes can be used. Confirmation can be directly off the selective agar if well isolated colonies are available.

- **Confirmation of *Salmonella***
  Colonies (maximum of 20) of presumptive *Salmonella* (subcultured on to nutrient agar if necessary) are confirmed by appropriate biochemical tests, as detailed in AS 5013.10 (2022). Preliminary confirmation at the isolating laboratory should include polyvalent O and H antisera. Rapid biochemical identification kits described in AOAC 978.24, AOAC 989.12, AOAC 991.13 and AOAC 2017.09 can be used for biochemical confirmation (section 9.5.3 of AS 5013.10). *Salmonella* isolates must be sent to a reference laboratory for serotyping.

CHECKLIST

Pre-enrichment	Is the buffered peptone water warmed to room temperature (to 36 ± 2°C for large quantities)?	
	Is the correct amount of enrichment broth used for the weight of sample analysed?	
	Is primary enrichment at 37 ± 1°C for 16-20h?	
	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 RVS broth sterilised at 115 °C for 15 minutes?	
	Is MKTTn broth boiled not autoclaved?	
	Is RVS incubated at 41.5 ± 1°C for 24 ± 3 h?	
	Is MKTTN incubated at 37 ± 1°C for 24 ± 3 h?	
	Are all complete selective liquid media prepared on the day of use (or is a validated shelf-life provided by the manufacturer)?	
Selective plating	What agars are used for isolation of suspect colonies?	
	Is the isolation of H_2S negative strains considered in the laboratories methods manual and procedures?	
Confirmation	How are cultures obtained for biochemical tests (if not streaked onto Nutrient agar is a purity check carried out)?	
	Are approved rapid bio-chemical test kits used?	
	Does preliminary confirmation at the isolating laboratory include polyvalent O and H antisera?	
	Are biochemical tests used sufficient to identify *Salmonella* spp.?	
	Are all suspect *Salmonella* sent to a reference Laboratory to be serotyped?	