Australian Government
Department of Agriculture and Water Resources

Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Colony count at 30°C by the pour plate technique – AS 5013.5:2016

SCOPE

All foods (including raw meats and carcase rinses and swabs).

PRINCIPLES

Samples are prepared according to instructions for that product in the appropriate standards or Microbiological Manual for Sampling and Testing of Export Meat and Meat Products. In general, for meat and meat products a 1:10 dilution is prepared using appropriate diluent(s). Pour plates of appropriate decimal dilutions are prepared using plate count agar and incubated aerobically at 30 ± 1°C for 72 ± 3 h, unless otherwise specified.

- **Sample preparation and incubation**
  Samples are diluted 1:10 in peptone salt solution[1] or buffered peptone water[2]. Suspension is homogenized and large particles are allowed to settle for up to 15 min if necessary. Sample (1 mL) is dispensed into duplicate Petri dishes using separate sterile pipettes. About 12 to 15 ml of Plate Count Agar[3] (44 to 47°C) is poured into each dish and the inoculum and media mixed by gently rotating the dishes. Carcase sponges can be enumerated without further dilution. Once solidified the plates are inverted and incubated at 30 ±1°C for 72 ± 3 h. Plates can be overlayed with agar (4 ml of 1.2 to 1.8% agar) if spreading colonies are likely to be present in the samples.

- **Counting of colonies**
  All colonies are counted. Care should be taken to include very small colonies but exclude particulate matter or undissolved material. Doubtful colonies can be carefully examined using a magnification aid. The countable range is approximately 15 – 300.

[1] Peptone salt solution, enzymatic digestion of casein 1 g; sodium chloride 8.5 g; water 1000 mL

[2] Buffered peptone water, Enzymatic digest of animal tissues 10 g; sodium chloride 5 g; disodium hydrogen phosphate dodecahydrate 9 g; potassium dihydrogen phosphate 1.5 g; water 1000 mL

[3] Plate Count Agar, enzymatic digestion of casein 5 g; yeast extract 2.5 g; glucose anhydrous 1 g; agar 9-18 g; water 1000 mL

CHECKLIST

Inoculation	Is peptone salt solution or buffered peptone water used for preparation of samples and dilutions?	_____
	Are appropriate dilution used to ensure a counting range of 15 to 300?	_____
	Is Plate Count Agar stored for no more than 3-months at 5 ± 3°C?	_____
	How is the shelf-life of prepared media controlled?	_____
	Is agar stabilised at 44 to 47°C prior to preparation of pour plates?	_____
	What volume of agar is used to prepare pour plates?	_____
	Are pour plates prepared correctly (check volume and mixing of sample)	_____
	Are separate sterile pipettes used to dispense 1 mL of the test suspension to each Petri dish?	_____
Incubation	What are the incubation conditions and period?	_____
	Are plates stored in stacks of <6?	_____
Interpretation	How are counts outside the countable range reported?	_____
	Is magnification used to aid in counting?	_____