Department	Micro Laboratory		Document no	MICLAB – METHOD 014	
Title	Presence or Absence Test of Salmonella SPP				
Prepared by:		Date:		Supersedes:	
Checked by:		Date:		Date Issued:	
Approved by:		Date:		Review Date:	

1. SCOPE AND APPLICATION

This general test method shall be followed in order to determine the presence or absence of Salmonella spp. This procedure applies to all products manufactured at a GMP site and the raw materials used to manufacture those products.

2. REAGENTS AND MATERIALS REQUIRED

2.1 Buffered peptone water (BPW) (OXOID CM 509)

2.2 Stomacher bags (Sterile)

2.3 Mannitol Selenite Cystine broth (MSC) (OXOID CM 399) or bio Merieux (42052)

2.4 Rappaport - Vassiliadis broth (RV) (OXOID CM 669)

2.5 Xylose Lysine Desoxycholate plates (XLD) (OXOID CM 469)

2.6 Bismuth Sulphite agar plates (BSA) (OXOID CM 201)

2.7 Streaking loops - 10□L

2.8 Homogeniser

2.9 Reference culture S. salford I.M.V.S. 1710

2.10 Reference culture C. freundii N.C.T.C. 9750

3. GENERAL TEST PROCEDURE

3.1 Weigh out 10g of sample into a stomacher bag.

3.2 Make up to 100g with BPW, or appropriate volume as pre determined by validation.

3.3 Place in homogeniser to mix (Pre-set for 15 seconds)

3.4 Allow to stand for approximately 1 hour.

3.5 The pH of the primary enrichment must be between 6.5-7.5.

For new products and raw materials, check pH. If not within 6.5-7.5, adjust using sterile 1N HCl and 1N NaOH.

3.6 Set up controls parallel with the test.
Note: Set up controls in Biohazard cabinet.

Using 2 MaCartney bottles of BPW (10mL), inoculate with C. freundii (-ve control) and S. salford (+ve control) respectively.

3.7 Incubate primary enrichment at 37 ± 1□C for 16-20 hours.

3.8 Remove from incubator - mix well and subculture primary enrichment broth into selective enrichment broths.

- 1 mL into 10 mLs of MSC (Mannitol Selenite Cystine broth containing 0.1mL of 0.1% filter sterilised L-cystine solution) pre-warmed to room temperature.
  NOTE: L-cystine solution is not added to MSC purchased from bio Merieux.

- 0.1 mL into 10 mL of RV (Rappaport-Vassiliadis) pre-warmed to room temperature.

- Vortex to mix.

3.9 Incubate MSC at 37 ± 1□C for 18-24 hours.
Incubate RV at 42 ± 1□C for 18-24 hours.

Note: Examine tubes for evidence of growth (turbidity and colour change) MSC from clear to red, RV from clear blue-green to turbid light blue-green.

3.10 Streak each broth onto selective agar plates XLD and BSA.

3.11 Incubate XLD plates in an inverted position for 24 hours at 37 ± 1□C.
Incubate BSA plates in an inverted position for 48 hours at 37 ± 1□C.

3.12 Remove plates from incubator and examine for typical colonies ie. red colonies with black centres on XLD and black (rabbit-eye or uniformly black) colonies with a black zone and metallic sheen on BSA.

NOTE: Routinely confirm suspect colonies by streaking onto TSA and following the appropriate steps outlines in OI150102.3.

The following describes the confirmatory steps outlined by the Australian Standards AS1766.2.5 - 1991.

3.13 Confirm at least 3 suspect colonies.

- Subculture each colony into a separate tube of peptone water (1%).
- Incubate at 37 ± 1□C. for 3 to 4 hours or until growth is visible.

- From each peptone water culture, inoculate the following media.

  i) Lysine decarboxylase broth
  ii) Lysine decarboxylase broth base (control).
  iii) ONPG broth
  iv) CLED agar plate - to check purity
  v) Nutrient agar slope.

3.14 Incubate all cultures at 37 ± 1□C. for 18-24 hours.

3.15 Examine all cultures and proceed as follows -

i) The purple Lysine decarboxylase broth remains purple after 18 hours. This reaction is typical of Salmonella.

Lysine decarboxylase broth base turns yellow and remains yellow. This reaction is atypical of Salmonella.

ii) ONPG broth remains colourless due to a negative beta-D-galactoside reaction. This is typical of Salmonella.

ONPG broth turns yellow due to a positive beta-D-galactoside reaction. This is atypical of Salmonella.

3.16 Serological confirmation.

i) Wash off growth ex nutrient slope with approx. 1 ml of formalinised saline solution.

ii) Mark three sections onto a glass slide. Place one drop of this suspension onto each section of a glass slide.

iii) Add one small drop of polyvalent 'O' antiserum to the first section and of polyvalent "H" antiserum to the second section.

iv) Tilt the slide back and forth for 1 minute and examine for agglutination. Agglutination in sections containing antisera indicates a positive result.

Agglutination in the third section indicates auto-agglutination and invalidates the test.

3.17 Interpretation of test results

- Isolates which give a result typical of salmonellae in both biochemical tests and serological reactions, are considered to be salmonellae.
- Isolates which give a result typical of salmonellae in one biochemical test and give negative serological results are considered not be salmonellae.
- Isolates which give typical reactions in both biochemical test but negative serological reactions and isolates which give a typical result in one biochemical test and positive serological reactions require further testing.

3.18 Carry out negative and positive controls throughout the test using the following reference cultures:

Reference Cultures

Salmonella Salford - IMVS 1710
Citrobacter freundii - NCTC 9750

4. REVIEW HISTORY

Version #	Revision History
MICLAB – METHOD 014	New

