SALMONELLAE & SHIGELLA

LIM P. Y.

INTRODUCTION

The presence in foods of any serotype of Salmonella is potentially dangerous as a source of human disease, either directly upon consumption of food, or indirectly through secondary contamination of utensils, processing equipments or processed foods. A further risk arises through induction of the carrier state in food-handlers.

I CULTURE MEDIA*

Nutrient broth
Selenite broth
Tetrathionate broth
Desoxycholate citrate agar (DCA)
Xylose lysine deoxycholate (XLD)
Triple sugar iron agar (TSI)
MacConkey agar (MCA)
GN broth
Salmonella anti-sera: Polyvalent "O" (somatic)
Polyvalent "H" specific and non-specific (flagellar)

* Refer to Appendix B for methods of media preparation.

II APPARATUS

'Waring' blender & flasks
Pipettes
Scissors & forceps
Alcohol (70% v/v) swabs
Plating loops
Inoculating needle
Conical flasks or screw-cap jars, 250 ml
Autoclave
Incubator
Agitated water bath
Weighing balance
Laminar flow chamber
Glass slides
Petri dish (90 × 15 mm)

III SAMPLING PROCEDURE

Refer to "AEROBIC PLATE COUNT" (E-2) Section III

IV PROCEDURE

A. RESUSCITATION (PRE-ENRICHMENT)

1. Weigh 50 g of the above sample and put them into a 'Waring' blender flask and add approximately 200 ml of sterile nutrient broth. Homogenise for 1 min at low speed.

   Also blend 50 g of above sample with 200 ml of GN broth for Shigella.

2. Incubate at 35°C for 24 hrs; for Shigella incubate at 35°C for 18 hrs.

B. SELECTIVE ENRICHMENT

1. Mix the resuscitated culture gently and add 1 ml each to 10 ml of tetrathionate broth and 10 ml of selenite broth.

2. Incubate the selective enrichment broths at 35°C for 24 hrs.

C. PLATING ON SELECTIVE AGAR MEDIA

1. Each culture of enrichment medium is inoculated onto DCA and XLD agar plates. Inoculate the same for Shigella from GN broth culture (from Step A-2) on MCA, DCA and XLD agar plates.

2. Transfer a loopful of culture and streak to obtain isolated colonies.

3. Incubate at 35°C for 24 hrs.

4. Examine the plates for the presence of Salmonella & Shigella colonies.

   For Salmonella:

   a) On XLD agar: appear as pink colonies with black centres of H_2S.

   b) On DCA agar: appear as colourless colonies.

   For Shigella:

   a) On XLD agar: appear as red or pink colour colonies, about 1 mm ∅.

   b) On DCA & MCA: appear as opague or transparent colonies.

D. SCREENING AND BIOCHEMICAL TESTS

1. Pick a suspected colony with inoculating wire and inoculate the TSI agar slant by streaking the slant and stabbing the butt. Incubate at 35°C for 24 hrs.

2. Salmonella cultures typically produce an alkaline (red) slant and acid (yellow) butt, with or without production of H_2S (blackening of butt) in TSI agar. Shigella cultures typically produce red slant and yellow butt, with no H_2S or gas.

3. Purify TSI cultures by streaking onto MCA and incubate for 24 hrs at 35°C. Typical colonies appear transparent and colourless, sometimes with a dark centre.

4. Subculture Salmonella colony in nutrient broth and incubate at 35°C for 24 hrs. Screen typical Shigella cultures in urea agar and motility medium. Shigella is urease negative and non-motile.

5. Using the nutrient broth culture as inoculum perform the following biochemical tests.

Tests (Salmonella)	Results	Tests (Shigella)	Results
Lysine decarboxylase	+	Glucose (gas)	−
Urease	−	VP	−
Dulcitol	+	MR	+
KCN	−	Indole	+/−
Malonate	−	Lysine	−
Indole	−	Arginine	+/−
VP	−	Ornithine	+/−
MR	+	Citrate	−
Citrate	+	Mannitol	+/−
Lactose	−	Lactose	−
Sucrose	−		

6. Incubate the tests for 24-28 hrs at 35°C.

7. Note that a large percentage of Salmonella arizonae strains are negative for dulcitol utilization; positive for malonate and lactose utilization.

8. Perform serological tests for cultures giving reactions typical of Salmonellae & Shigella.

E. SEROLOGICAL CONFIRMATION

1. Emulsify the culture in 2 drops of saline on a clean glass slide.

2. Add one some loopful of polyvalent "O" antiserum to the first drop only. Use the second drop as a saline control.

3. Tilt the slide back and forth for 1 minute and examine for agglutination. A positive reaction is when there is agglutination in the test mixture but not in the saline control.

4. Repeat similarly with polyvalent "H" antiserum.

5. Salmonella isolates causes agglutination for both antisera.

6. Conduct the serology for Shigella from Step 1 to Step 3.

FLOW DIAGRAM OF EXAMINATION PROCEDURES FOR SALMONELLA

50 g sample + 200 ml nutrient broth
(Pre-enrichment)
35°C/24 hrs

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Enrichment in

a) Selenite broth (35°C/24 hrs)

b) Tetrathionate broth (35°C/24 hrs)

↓

Streak onto

a) Desoxycholate citrate agar (DCA)

b) Xylose lysine deoxycholate (XLD)

↓

35°C/24 hrs

↓

Typical colonies

↓

Streak onto TSI

↓

Confirmatory tests:

a) Lysine decarboxylase
b) Urease
c) Dulcitol
d) Malonate
e) KCN
f) Indole
g) VP
h) MR
i) Citrate
j) Lactose
k) Sucrose

↓

Serology
(Polyvalent "O" antiserum, "H" antiserum)

FLOW DIAGRAM OF EXAMINATION PROCEDURES FOR SHIGELLA

50 g sample + 200 ml GN broth
35°C/18 hrs

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Streak onto

1) MCA

2) DCA

3) XLD

35°C/24 hrs

↓

TSI

35°C/24 hrs

↓

if alkaline/acid; no H_2S, no gas

urease: –

motility: –

↓

Confirmatory tests:

Glucose (gas)	Arginine
VP	Ornithine
MR	Citrate
Indole	Mannitol
Lysine	Lactose

↓

Serology