SCHEDULE 2

Testing Methods

PART 2

METHODS FOR THE ISOLATION OF *SALMONELLA*

A. BACTERIOLOGICAL METHOD

1. Tests shall be begun on receipt of the sample or on the first working day which allows this method to be completed. If the test is not begun on the day of receipt the sample shall be stored in a refrigerator until required. If the sample has been refrigerated it shall be removed from the refrigerator and stored at room temperature for at least four hours before the test is started.

Day 1

2. Tests shall be carried out in duplicate using two 25 gram portions of each sample submitted for testing. Each 25 gram sample shall be placed aseptically in a container containing 225 ml Buffered Peptone Water (BPW) and incubated at 37°C for 18 hours±2 hours.

Day 2

3. 0.1 ml from the jar of incubated BPW shall be inoculated into 10 ml Rappaports Vassiliadis broth (RV broth)(**1**) and incubated at 41.5°C±0.5°C for 24 hours.

Day 3

4. The RV broth shall be plated out on to two 90 millimetre plates of Brilliant Green Agar (BGA)(**2**), or on to one 90 millimetre plate of BGA and one 90 millimetre plate of Xylose Lysine Deoxycholate Agar (XLD)(**3**), using a 2.5 mm diameter loop. The plates shall be inoculated with a droplet taken from the edge of the surface of the fluid by drawing the loop over the whole of one plate in a zig zag pattern and continuing to the second plate without recharging the loop. The space between the loop streaks shall be 0.5 cm – 1.0 cm. The plates shall be incubated at 37°C±2°C for 24 hours ± 3 hours.

5. The residual RV broth shall be reincubated at 41.5°C±0.5°C for a further 24 hours.

Day 4

6. The plates shall be examined and a minimum of 3 colonies from each plate showing suspicion of *Salmonella* growth shall be subcultured–

(a) on to a blood agar plate;

(b) on to a MacConkey agar plate(**4**); and

(c) into biochemical media suitable for the identification of *Salmonella*.

(**1**) Rappaports Vassiliadis Broth – see Vassiliadis P, Pateraki E, Papaiconomou N, Papadkis J A, and Trichopoulos D (1976) Annales de Microbiologie (Institut Pasteur) 127B: 195–200. Elsevier, 23 rue Linois, 75724 Paris, Cedex 15, France.

(**2**) Brilliant Green Agar – see Edel W and Kampelmacher E H (1969) Bulletin of World Health Organisation 41:297–306, World Health Organisation Distribution and Sales, CH-1211, Geneva 27, Switzerland (ISSN 0042-9686).

(**3**) Xylose Lisene Deoxycholate Agar – see Taylor W I, (1965) American Journal of Clinical Pathology, 44:471–475, Lippincott and Raven, 227E Washington Street, Philadelphia PA 19106, USA.

(**4**) MacConkey agar – see (1963) International Standards for Drinking Water, World Health Distribution and Sales, CH 1211, Geneva 27, Switzerland.

These media shall be incubated at 37°C overnight.

7. The reincubated RV broth shall be plated out as described in paragraph 4.

Day 5

8. The incubated composite media or equivalent shall be examined and the findings recorded, discarding cultures which are obviously not *Salmonella*. Slide serological tests shall be performed using *Salmonella* polyvalent "O" and polyvalent "H" (phase 1 and 2) agglutinating sera on selected suspect colonies collected from the blood agar or MacConkey plates. If reactions occur with one or both sera, the colonies shall be typed by slide serology and a subculture sent to a veterinary laboratory nominated in writing by the Scottish Ministers for this purpose for further typing.

9. The plates referred to in paragraph 7 shall be examined and further action taken as in paragraph 6 and 8.

B. ELECTRICAL CONDUCTANCE METHOD

1. Tests shall be begun on receipt of the sample or on the first working day which allows the following method to be completed. If the test is not begun on the day of receipt the sample shall be stored in a refrigerator until required. If the sample has been refrigerated it shall be stored at room temperature for at least four hours before the test is started.

Day 1

2. Tests shall be carried out in duplicate using two 25 gram portions of each sample submitted for testing. Each 25 gram sample shall be placed aseptically in a sterile container containing 225 ml Buffered Peptone Water/Lysine/Glucose (BPW/L/G)(**5**) and incubated at 37°C for 18 hours.

Day 2

3. The incubated BPW/L/G shall be added to Selenite Cystine Trimethylamine-N-Oxide Dulcitol (SC/T/D)(**6**) and Lysine Decarboxylase Glucose (LD/G)(**7**) media in electrical conductance cells or wells. For cells or wells containing more than 5 ml medium 0.2 ml of the BPW/L/G shall be added and for cells or wells containing 5 ml or less medium 0.1 ml of the BPW/L/G shall be added. Cells or wells shall be connected to appropriate electrical conductance measuring equipment set to monitor and record changes in electrical conductance at 6 minute intervals over a 24 hour period. The temperature of cells and wells shall be kept at 37°C.

Day 3

4. At the end of the 24 hour period, the information recorded by the conductance measuring equipment shall be analysed and interpreted using criteria defined by the manufacturers of the equipment. Where a well or cell is provisionally identified as being positive for *Salmonella*, the result shall be confirmed by subculturing the contents of the well or cell on to two 90 millimetre plates of BGA or on to one 90 millimetre plate of BGA and one 90 millimetre plate of Xylose Lysine Deoxycholate Agar (XLD) using a 2.5 mm diameter loop. The plates shall be inoculated with a droplet taken from the edge of the surface of the fluid by drawing the loop over the whole of one

(**5**) Buffered Peptone Water/Lysine/Glucose – see Ogden I D (1988) International Journal of Food Microbiology 7:287 297, Elsevier Science BV, PO Box 211, 1000 AE, Amsterdam, Netherlands (ISSN 0168-1695).
(**6**) Selenite Cystine Trimethylamine-N-Oxide Dulcitol – see Easter, M C and Gibson, D M, (1985) Journal of Hygiene 94:245–262, Cambridge University Press, Cambridge.
(**7**) Lysine Decarboxylase Glucose – see Ogden I D (1988) International Journal of Food Microbiology 7:287–297, Elsevier Science BV, PO Box 211, 1000 AE, Amsterdam, Netherlands (ISSN 0168-1695).

plate in a zig zag pattern and continuing to the second plate without recharging the loop. The space between the loop streaks shall be 0.5 cm – 1.0 cm. The plates shall be incubated at 37°C overnight.

Day 4

5. The plates shall be examined and a minimum of 3 colonies from each plate showing suspicion of *Salmonella* growth shall be subcultured–

(a) on to a blood agar plate;

(b) on to a MacConkey agar plate; and

(c) into biochemical media suitable for the identification of *Salmonella*.

These media shall be incubated at 37°C overnight.

Day 5

6. The incubated composite media or equivalent shall be examined and the findings recorded, discarding cultures which are obviously not *Salmonella*. Slide serological tests shall be performed using *Salmonella* polyvalent "O" and polyvalent "H" (phase 1 and 2) agglutinating sera on selected suspect colonies collected from the blood agar or MacConkey plates. If reactions occur with one or both sera, a subculture shall be sent to a veterinary laboratory nominated in writing by the Scottish Ministers for this purpose for further typing.