SCHEDULE 3

Testing Methods

PART II

METHODS FOR THE ISOLATION OF SALMONELLA

A.

BACTERIOLOGICAL METHOD

1.—(1) Tests must 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 must be stored in a refrigerator until required. If the sample has been refrigerated it must be removed from the refrigerator and stored at room temperature for at least four hours before the test is started.

(2) Tests must be carried out in duplicate using two 25 gram portions of each sample submitted for testing.

Day one

2. On day one, each 25 gram sample must be placed aseptically in a container containing 225 ml Buffered Peptone Water (BPW) and incubated at 37°C±1°C for 18 hours±2 hours.

Day two

3. On day two, 0.1 ml from the container of incubated BPW must be inoculated into 10 ml Rappaports Vassiliadis broth (RV broth)(**1**) and incubated at 41.5°C±0.5°C for 24 hours ± 3 hours.

Day three

4. On day three, the RV broth must 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 must 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 must be 0.5 cm – 1.0 cm. The plates must be incubated at 37°C ±2°C for 24 hours ± 3 hours.

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

Day four

6. On day four the plates must be examined and a minimum of 3 colonies from each plate showing suspicion of Salmonella growth must be subcultured–

(a) on to a blood agar plate;

(**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.

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

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

These media must be incubated at 37°C overnight.

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

Day five

8. On day five the incubated composite media or equivalent must be examined and the findings recorded, discarding cultures which are obviously not Salmonella. Slide serological tests must 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 must be typed by slide serology. If requested in writing by the National Assembly , the operator of the laboratory must send a subculture to a Regional Veterinary Laboratory of the Veterinary Laboratories Agency of the Department for Environment, Food and Rural Affairs for further typing.

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

B.

ELECTRICAL CONDUCTANCE METHOD

10. Tests must 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 must be stored in a refrigerator until required. If the sample has been refrigerated it must be stored at room temperature for at least four hours before the test is started.

Day one

11. On day one tests must be carried out in duplicate using two 25 gram portions of each sample submitted for testing. Each 25 gram sample must 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 two

12. On day two the incubated BPW/L/G must 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 must be added and for cells or wells containing 5 ml or less medium 0.1 ml of the BPW/L/G must be added. Cells or wells must 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 must be kept at 37°C.

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

(**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).

Day three

13. On day three, at the end of the 24 hour period, the information recorded by the conductance measuring equipment must 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 must 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 must 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 must be 0.5 cm – 1.0 cm. The plates must be incubated at 37°C overnight.

Day four

14. On day four the plates must be examined and a minimum of 3 colonies from each plate showing suspicion of Salmonella growth must 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 must be incubated at 37°C overnight.

Day five

15. On day five the incubated composite media or equivalent must be examined and the findings recorded, discarding cultures which are obviously not Salmonella. Slide serological tests must 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 must be typed by slide serology. If requested in writing by the National Assembly , the operator of the laboratory must send a subculture to a Regional Veterinary Laboratory of the Veterinary Laboratories Agency of the Department for Environment, Food and Rural Affairs for further typing.