SCHEDULE 2

TESTING METHODS

PART II

METHODS FOR THE ISOLATION OF*salmonella*

A. BACTERIOLOGICAL METHOD

20. 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 one hour before the test is started.

Day 1

21. Tests shall be carried out in duplicate using two 25 gram ± 1 gram portions of each sample submitted for testing. Each 25 gram ± 1 gram sample shall be placed aseptically in a container containing 225 ml ± 1 ml Buffered Peptone Water (BPW) and incubated at 37°C ± 1°C for 18 hours ± 2 hours.

Control Tests

22. Control tests shall be carried out each day that a test is initiated using –

(a) *Salmonella java*NCTC 5706 or equivalent no more than 28 days old at the time of use;

(b) *Erwinia herbicola* NCTC 9381 or equivalent not more than 28 days old at the time of use; and

(c) processed animal protein or compost or digestion residue which is free of *Salmonella java*.

23. 10 gram ± 1 gram portions of the rendered animal protein shall be placed aseptically in each of two sterile containers containing 90 ml ± 1 ml Buffered Peptone Water (BPW)(**1**) and mixed thoroughly until the samples are evenly suspended.

24. One colony of *Salmonella java* (22)(a) shall be placed in 10 ml ± 1 ml BPW and mixed to form an even suspension. 0.1 ml of the suspension shall be added to the suspension in the preceding paragraph. This shall be repeated for *Erwinia herbicola* (22)(b).

25. These are then treated and examined in the same way as test samples. If no typical colonies are formed then that day's testing shall be invalid and shall be repeated.

Day 2

26. 0.1 ml from the container of incubated BPW shall be inoculated into 10 ml ± 1 ml Rappaport-Vassiliadis Soya Broth (RVS broth)(**2**) and incubated at 41.5°C ± 0.5°C for 18 to 24 hours.

(**1**) Buffered Peptone Water – *See* Edel, W. and Kampelmacher, E.H. (1973) Bulletin of World Health Organisation, 48: 167-174, World Health Organisation Distribution and Sales, CH-1211, Geneva 27, Switzerland (ISSN 0042-9686)

(**2**) Van Schothurst M., Renauld A., and Van Beek C. (1987) Food Microbiology 4: 11-18.

Day 3

27. The RVS broth shall be plated out onto two 90 mm plates of Brilliant Green Agar (BGA)(**3**) or onto one 90 mm plate of BGA and one 90 mm plate of Xylose Lysine Deoxycholate Agar (XLD)(**4**) 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 ± 1°C for 18 to 24 hours.

28. The residual RVS broth shall be reincubated at 41.5°C ± 0.5°C for a further 18 to 24 hours.

Day 4

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

(a) onto a nutrient agar plate;

(b) onto a MacConkey agar plate(**5**); and

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

These media shall be incubated at 37°C ± 1°C overnight.

30. The reincubated RVS broth shall be plated out as described in paragraph 27.

Day 5

31. 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 nutrient agar or MacConkey agar plates. If reactions occur with one or both sera, a subculture of the colonies shall be sent to one of the Department's laboratories at either Agriculture, Food and Environmental Science Division, Newforge Lane, Belfast, BT9 5PX or, Veterinary Science Division, Stoney Road, Belfast, BT4 3SD, for further typing.

32. The plates referred to in paragraph 30 shall be examined and further action taken as in paragraphs 29 and 31.

B. ELECTRICAL CONDUCTANCE METHOD

33. Tests shall be begun on receipt of the samples 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 removed from the refrigerator and stored at room temperature for at least one hour before the test is started.

Day 1

34. Tests shall be carried out in duplicate using two 25 gram ± 1 gram portions of each sample submitted for testing. Each 25 gram ± 1gram sample shall be placed aseptically in a sterile container

(**3**) 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).

(**4**) Xylose Lysine Deoxycholate Agar – *See* Taylor, W.I. (1965) American Journal of Clinical Pathology, 44:471-475, Lippincott and Raven, 227 E. Washington Street Philadelphia PA19106, USA

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

containing 225 ml ± 1 ml Buffered Peptone Water (BPW) (**6**) and incubated at 37°C ± 1°C for 18 to 24 hours.

Control Tests

35. Control tests shall be carried out each day that a test is initiated using –

(a) *Salmonella java* NCTC 5706 or equivalent no more than 28 days old at the time of use;

(b) *Erwinia herbicola* NCTC 9381 or equivalent not more than 28 days old at the time of use; and

(c) processed animal protein or compost or digestion residue which is free of *Salmonella java*.

36. 10 gram ± 1 gram portions of the rendered animal protein shall be placed aseptically in each of two sterile containers containing 90 ml ± 1 ml Buffered Peptone Water (BPW)(**6**) and mixed thoroughly until the samples are evenly suspended.

37. One colony of *Salmonella java* (35)(a) shall be placed in 10 ml ± 1 ml BPW and mixed to form an even suspension. 0.1 ml of the suspension shall be added to the suspension in the preceding paragraph. This shall be repeated for *Erwinia herbicola* (35)(b).

38. These are then treated and examined in the same way as test samples. If no typical colonies are formed then that day's testing shall be invalid and shall be repeated.

Day 2

39. The incubated BPW shall be added to Rappaport -Vassiliadis Soya (RVS) Broth in tubes to be inserted into electrical conductance cells. Detection of growth will utilise indirect impediometry as in the method of Donaghy and Madden (1993)(**7**). For cells or tubes containing more than 5 ml (RVS) medium 0.2 ml of the BPW shall be added and for cells or tubes containing 5 ml or less (RVS) medium 0.1 ml of the BPW shall be added. Cells or tubes 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 tubes shall be kept at 42°C ± 0.5°C.

Day 3

40. 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 tube or cell is provisionally identified as being positive for *Salmonella*, the result shall be confirmed by subculturing the contents of the tube or cell onto two 90 mm plates of BGA or onto one 90 mm plate of BGA and one 90 mm 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 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 ± 1°C overnight.

Day 4

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

(**6**) Buffered Peptone Water – *See* Edel, W. and Kampelmacher, E. H. (1973) Bulletin of World Health Organisation, 167-174, World Health Organisation Distribution and Sales, CH-1211, Geneva 27, Switzerland (ISSN 0042-9686)

(**6**) Buffered Peptone Water – *See* Edel, W. and Kampelmacher, E. H. (1973) Bulletin of World Health Organisation, 167-174, World Health Organisation Distribution and Sales, CH-1211, Geneva 27, Switzerland (ISSN 0042-9686)

(**7**) Donaghy and Madden – *See* Donaghy, J. A. and Madden R. H. (1993) International Journal of Food Microbiology. 17; 281-288

(a) onto a nutrient agar plate;

(b) onto a MacConkey agar plate; and

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

These media shall be incubated at 37°C ± 1°C overnight.

Day 5

42. 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 nutrient agar or MacConkey agar plates. If reactions occur with one or both sera, a subculture of the colonies shall be sent to one of the Department's laboratories at either Agriculture, Food and Environmental Science Division, Newforge Lane, Belfast, BT9 5PX, or Veterinary Science Division, Stoney Road, Belfast, BT4 3SD, for further typing.