SCHEDULE 3

Sampling and Testing Methods

Part III

Methods for the Isolation of Salmonella

B. Electrical conductance method

1. Tests shall be begun on receipt of the samples of processed animal protein 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 four hours before the test is started.

Day 1

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

Day 2

3. The incubated BPW shall be added to Rappaport Vassiliadis Broth in electrical conductance cells or tubes to be inserted into electrical conductance cells. Detection of growth will utilise indirect impediometry as in the method of Donaghy and Madden (1993)(**1**). For cells or tubes containing more than 5 ml medium 0.2 ml of the BPW shall be added and for cells or tubes containing 5ml or less 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.

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 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 90mm 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

5. 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; and

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

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

These media shall be incubated at 37°C ± 1°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 nutrient agar or MacConkey plates. If reactions occur with one or both sera, the colonies shall be typed by slide serology and a subculture shall be sent to one of the Department's laboratories at either Food Science Division, Newforge Lane, Belfast, BT9 5PX or Veterinary Science Division, Stoney Road, Belfast, BT4 3SD, for further typing.