SCHEDULE 1

Article 3(b) and 4

PART II

BACTERIOLOGICAL METHODS

Bacteriological method (Rappaports) for the detection of salmonella in chick box liners, cloacal swabs, composite faeces samples and carcases.

Samples submitted for testing for the presence of salmonella shall be examined in the following prescribed manner on consecutive days and where a laboratory at which samples have been received for testing on any day is unable to commence such an examination on that day, the samples shall be stored in a refrigerator at between 1°C and 4°C until required for examination.

Day 1

(a) Chick box liners: a one gram portion shall be taken from a soiled area on each liner and the portions from separate liners shall be bulked together and placed in Buffered Peptone Water (BPW)(a), at the rate of 1 gram of liner in 10 ml of BPW up to a maximum of 10 grams in 100 ml of BPW.

(b) Faeces samples: the composite faeces sample shall be thoroughly mixed and a sub-sample weighing not more than 10 grams shall be placed in BPW at the rate of 1 gram faeces to 10 ml BPW up to a maximum of 10 grams in 100 ml BPW.

(c) Cloacal swabs: cloacal swabs shall be bulked together in batches and placed in BPW at the rate of 1 swab to 4 ml BPW up to a maximum of 30 swabs in 120 ml BPW.

(d) Carcases of birds: the following organs shall be removed from the carcases of birds –

(i) from chicks – samples of the yolk sac, liver and terminal intestines (to include portions of small intestines, large intestines and caecal tonsil).

(ii) from birds of 4 weeks of age – samples of liver and terminal intestines (to include portions of small intestines, large intestine and caecal tonsil).

The samples of organs taken from the carcases of birds submitted shall then be bulked together and placed in BPW at the rate of 1 gram of bulked tissue in 10 ml BPW up to a maximum of 10 grams of tissue in 100 ml BPW.

The inoculated BPW shall then be incubated at 37°C for 18–24 hours.

Day 2

0.1 ml from the incubated BPW shall be inoculated into 10 ml of Rappaports Vassiliadis (RV) broth (b) and incubated at 42.5°C for 18–24 hours.

Day 3

The RV broth shall be plated out on to two plates of Brilliant Green Agar (BGA) (d) using a 2.5 mm diameter loop. The BGA plates shall be inoculated with a droplet taken from the edge of the surface of the fluid and drawing the loop over the whole of one plate in a zigzag 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 for 18–24 hours, and the RV broth reincubated at 42.5°C for a further 18–24 hours.

Day 4

(i) The plates of BGA shall be examined and a minimum of 3 colonies from the plates showing suspicion of salmonella growth shall be subcultured on to a blood agar plate

and a MacConkey agar plate and into biochemical composite media or equivalent. These media shall be incubated at 37°C for 18–24 hours.

(ii) The reincubated RV broth shall be plated out, and the plates incubated, as described in Day 3.

Day 5

(i) The incubated plates and 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" (Groups A–S) 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 to Group level by slide serology.

(ii) The plates of BGA prepared at Day 4 (ii) shall be examined and further action taken as described in Day 4 (i) and Day 5 (i).

Bateriological method (Selenite) for the detection of salmonella in chick box liners, cloacal swabs, composite faeces samples and carcases.

Samples submitted for testing for the presence of salmonella shall be examined in the following prescribed manner on consecutive days and where a laboratory at which samples have been received for testing on any day is unable to commence such an examination on that day, the samples shall be stored in a refrigerator at between 1°C and 4°C until required for examination.

Day 1

(a) Chick box liners: a one gram portion shall be taken from a soiled area on each liner and the portions from separate liners shall be bulked together and placed in Selenite F broth (c) at the rate of 1 gram of liner to 10 ml broth up to a maximum of 10 grams of liner in 100 ml broth.

(b) Faeces samples: the composite faeces sample shall be thoroughly mixed and a sub-sample weighing not more than 10 grams shall be placed in Selenite F broth at the rate of 1 gram of faeces to 10 ml broth up to a maximum of 10 grams of faeces in 100 ml broth.

(c) Cloacal swabs: cloacal swabs shall be bulked together in batches and placed in Selenite F broth at the rate of 1 swab to 4 ml broth up to a maximum of 30 swabs in 120 ml broth.

(d) Carcases of birds: the following organs shall be removed from the carcases of birds –

(i) from chicks – samples of the yolk sac, liver and terminal intestines (to include portions of small intestines, large intestine and caecal tonsil).

(ii) from birds of 4 weeks of age – samples of liver and terminal intestines (to include portions of small intestines, large intestine and caecal tonsil).

The samples of organs taken from the carcases of birds submitted shall then be bulked together and placed in Selenite F broth at the rate of 1 gram of bulked tissue in 10 ml of broth up to a maximum of 10 grams of tissue in 100 ml broth.

The inoculated Selenite F broth shall then be incubated at 37°C for 18–24 hours.

Day 2

(i) The Selenite F broth shall be plated out on to two plates of Brilliant Green Agar (BGA) (d) using a 2.5 mm diameter loop. The BGA plates shall be inoculated with a droplet taken from the edge of the surface of the fluid and drawing the loop over the whole of one plate in a zigzag 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 for 18–24 hours.

(ii) The Selenite F broth shall then be reincubated at 37°C for a further 18 –24 hours.

Day 3

(i) The plates of BGA shall be examined and a minimum of 3 colonies from the plates showing suspicion of salmonella growth shall be subcultured on to a blood agar plate and a MacConkey agar plate and into biochemical composite media or equivalent. These media shall be incubated at 37°C for 18–24 hours.

(ii) The reincubated Selenite F broth shall be plated out and incubated as described in Day 2 (i).

Day 4

(i) The incubated plates and 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" (Groups A–S) 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 to Group level by slide serology.

(ii) The plates of BGA prepared at Day 3 (ii) shall be examined and further action taken as described in Day 3 (i) and Day 4 (i).

(a) Buffered Peptone Water – Edel and Kampelmacher (1973) (Commercially available as Oxoid CM 509, Lab M46 or equivalent).

(b) Rappaports Vassiliadis (RV) Broth – Vassiliadis et al (1976) (Commercially available as Oxoid CM 669 or equivalent).

(c) Selenite F broth – Liefson (1936) (Commercially available as Oxoid CM 395 and L121, Lab M44a and 44b or equivalent).

(a), (b) and (c) should be reconstituted according to the manufacturer's instructions.

(d) Brilliant Green Agar (Modified) – Edel and Kampelmacher (1969) (Commercially available as Oxoid CM 329, Lab M34 or equivalent).

The agar should be reconstituted according to the manufacturer's instructions and poured into 9 cm diameter plates.

References:

Liefson E (1936) American Journal of Hygiene 24, 423–432.

Edel, W & Kampelmacher, E H (1969) Bulletin of the World Health Organisation 41, 297–306.

Edel, W & Kampelmacher, E H (1973) Bulletin of the World Health Organisation 48, 167–174.

Anon (1969) ISO 6579 International Organisation for Standardisation, Geneva.

Vassiliadis, P, Pateraki, E, Papaiconomou, N, Papadakis, J A and Trichopoulos, D (1976) Annales de Microbiologie (Institut Pasteur) 127B, 195–200.