SCHEDULE 2

Testing Methods

PART 3

METHOD FOR THE ISOLATION OF *ENTEROBACTERIACEAE*

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 at between 2°C and 8°C. 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.

Samples

2. Tests shall be carried out using five 10 gram portions of each sample submitted for testing. Each 10 gram sample shall be placed aseptically in a sterile container containing 90 ml Buffered Peptone Water and mixed thoroughly until the sample is evenly suspended.

Inoculations

3. For each portion of the sample 1 ml of solution shall be transferred to a sterile 90 mm petri dish (in duplicate). The plates shall be labelled to identify the portion of sample they were taken from. 15 ml of Violet Red Bile Glucose Agar (VRBGA)(**1**) at a temperature of 47°C±2°C shall be added to each petri dish and immediately gently mixed by swirling the dish with five clockwise and five anticlockwise circular movements.

4. Once the agar has set, each agar plate shall be overlaid with a further 10 ml VRBGA at a temperature of 47°C±2°C. Once the overlay has set, the plates shall be inverted and incubated aerobically at 37°C±1°C for 20 hours±2 hours.

Samples with colonies of *Enterobacteriaceae*

5. After incubation each set of duplicate plates shall be examined for colonies characteristic of *Enterobacteriaceae* (purple colonies 1 – 2 mm in diameter). All characteristic colonies on each plate shall be counted and the arithmetic mean of the duplicate plates taken.

The sample provisionally fails if either–

(a) any arithmetic mean is above 30(**2**); or

(b) three or more arithmetic means are above 10,

in which case the following procedure shall be followed to establish whether or not the colonies are *Enterobacteriaceae*.

6. After counting the colonies, characteristic colonies shall be taken at random from the agar plates, the number being at least the square root of the colonies counted. The colonies shall be subcultured onto a blood agar plate and incubated aerobically at 37°C±1°C for 20 hours±2 hours.

(**1**) Violet Red Bile Glucose Agar – see Mossell D A A, Eelderink I, Koopmans M, van Rossem F (1978) Laboratory Practice 27 No. 12 1049–1050; Emap Maclaren, PO Box 109, Maclaren House, 19 Scarbrook Road, Croydon CR9 1QH.

(**2**) An arithmetic mean of 30 is equivalent to 3x102 colony forming units per gram of original sample.

Examination of subcultures

7. An oxidase test and a glucose fermentation test shall be performed on each of the five subcultured colonies. Colonies which are oxidase-negative and glucose fermentation-positive shall be considered to be *Enterobacteriaceae*.

8. If not all of the colonies prove to be *Enterobacteriaceae*, the total count in paragraph 5 shall be reduced in proportion prior to establishing whether or not the sample should fail.

Controls

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

(a) *Escherichia coli*NCTC 10418 no more than seven days old at time of use; and

(b) processed animal protein or compost or digestive residue which is free of *Enterobacteriaceae*.

10. A 10 gram portion of the rendered animal protein shall be placed aseptically in a sterile container containing 90 ml BPW and mixed thoroughly until the sample is evenly suspended.

11. One colony of *Escherichia coli* shall be placed in 10 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.

12. This is 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.