SCHEDULE 2

TESTING METHODS

PART I

METHOD FOR THE ISOLATION OF*clostridium perfringens*

Examination of subcultures

Motility

10. The motility nitrate medium shall be examined for the type of growth along the stab line. If there is evidence of diffuse growth out into the medium away from the stab line, the bacteria shall be considered to be motile.

Reduction of nitrate to nitrite

11. After examination of the motility nitrate medium 0.2 ml to 0.5 ml of nitrite detection reagent shall be added to it. The formation of a red colour confirms that the bacteria have reduced nitrate to nitrite. Cultures that show a faint reaction (i.e. a pink colour) should be discounted. If no red colour is formed within 15 minutes, a small amount of zinc dust shall be added and the tube allowed to stand for approximately15 minutes. If a red colour is formed after the addition of zinc dust no reduction of nitrate to nitrite has taken place.

Production of gas and acid from lactose and liquefaction of gelatine

12. The lactose gelatine medium shall be examined for the presence of small gas bubbles in the medium.

13. The lactose gelatine medium shall be examined for colour. A yellow colour indicates fermentation of lactose.

14. The lactose gelatine medium shall be chilled for up to one hour at 2–8°C and then checked to see if the gelatine has liquefied. If the medium has solidified it shall be re-incubated anaerobically for a further 18-24 hours, the medium chilled for a further one hour at 2-8°C and again checked to see if the gelatine has liquefied.

15. The presence of *Clostridium perfringens* shall be determined on the basis of the results from paragraphs 10 to 14. Bacteria which produce black colonies on EY-free TSC agar, are non-motile, reduce nitrate to nitrite, produce gas and acid from lactose and liquefy gelatine within 48 hours shall be considered to be *Clostridium perfringens*.

Control Tests

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

(a) *Clostridium perfringens*NCTC 10662(**1**) no more than 28 days old at the time of use;

(b) *Escherichia coli* NCTC 10418 or equivalent not more than 28 days old at the time of use; and

(**1**) The National Collection of Type Cultures, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT.

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

17. 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)(**2**) and mixed thoroughly until the samples are evenly suspended.

18. One colony of *Clostridium perfringens* (16)(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 *Escherichia coli* (16)(b).

19. 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.

(**2**) 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)