ANAEROBIC PLATE COUNT
(Spread and Pour Plate Method)

MAKOTO YAMAGATA

INTRODUCTION

The anaerobic plate count provides an estimate of the number of viable anaerobic microorganisms in food and is affected by the type of medium used and the length and temperature of incubation.

The spread and pour plate methods described below are the same as those used in determining the aerobic plate count. The only difference is in the anaerobic incubation of the media.

APPARATUS

1. Anaerobic jar
2. Vacuum pump and nitrogen gas bomb, if necessary
3. BBL Gas pack plus anaerobic jar system and gas pack pouch (see instruction manual of BBL) is a convenient and easier method for anaerobic condition.
4. "Waring" blender & flasks or Stomacher ("Lab-blender" 400)
5. Pipettes, 1 ml, 5 ml
6. Scissors, scalpels & forceps
7. Alcohol (70%, v/v) swabs
8. Bent glass spreader
9. Incubator (35° ± 1°C)
10. Autoclave
11. Weighing balance
12. Laminar flow cabinet
13. Bunsen burner
14. Sterile petri dish (ø : 90 mm, H : 15 mm)

CULTURE MEDIA
(Refer to Appendix B for methods of media preparation)

1. Anaerobic agar

Trypticase peptone	17.5 g
Phytone peptone	2.5 g
Sodium chloride	2.5 g
L-cysteine	0.4 g
Dextrose	10.0 g
Agar	15.0 g
Sodium thioglycollate	2.0 g
Sodium formaldehyde sulfoxylate	1.0 g
Methylene blue	0.002 g
Distilled water	1 litre
Final pH	7.2 ± 0.1

Autoclave for 15 min at 121°C.

2. SPS agar

Sodium sulfite	0.50 g
Polymyxin sulfate	0.01 g
Sulfadiazine	0.12 g
Trypticase peptone	15.00 g
Yeast extract	10.00 g
Agar	13.90 g
Iron citrate	0.50 g
Distilled water	1 litre
Final pH	7.0 ± 0.1

Autoclave for 15 min at 118°C.

Suitable media for detection of *Clostridium perfringens* (welchii) in food. Black colonies are sulfite reducers.

3. TSN agar

Trypticase peptone	15.00 g
Sodium sulfite	1.00 g
Neomycin sulfite	0.02 g
Polymycin sulfite	0.05 g
Yeast extract	10.00 g
Ferric citrate	0.50 g
Agar	13.50 g
Final pH	7.2 ± 0.1

Autoclave for 15 min at 118°C.

Suitable media for detection of *Clostridium perfringens* (welchii) in food. Black colonies are sulfite reducers. 5 ml of buffered thioglycollate solution may be added to 200 ml of TSN agar at 77°C, if desired.

PROCEDURE

1. Sampling Procedure

   Same as in aerobic plate count method.

2. Sample Preparation

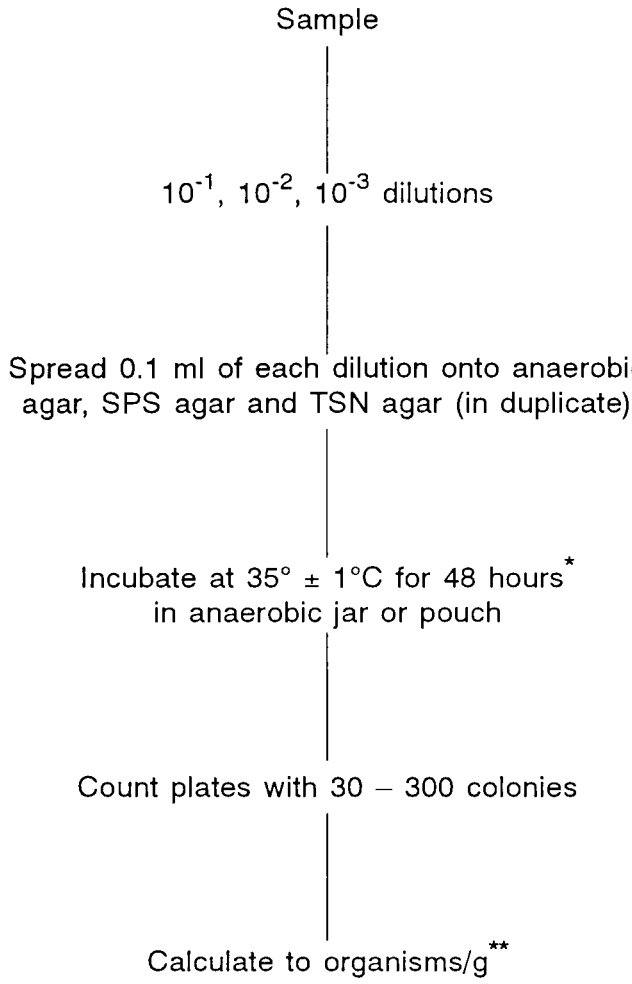
   Same as in aerobic plate count method.

3. Spread plate method (Fig. 1)

   Same as in aerobic plate count method, however culture media used are anaerobic agar (SPS agar and TSN agar).

4. Pour plate method (Fig. 2)

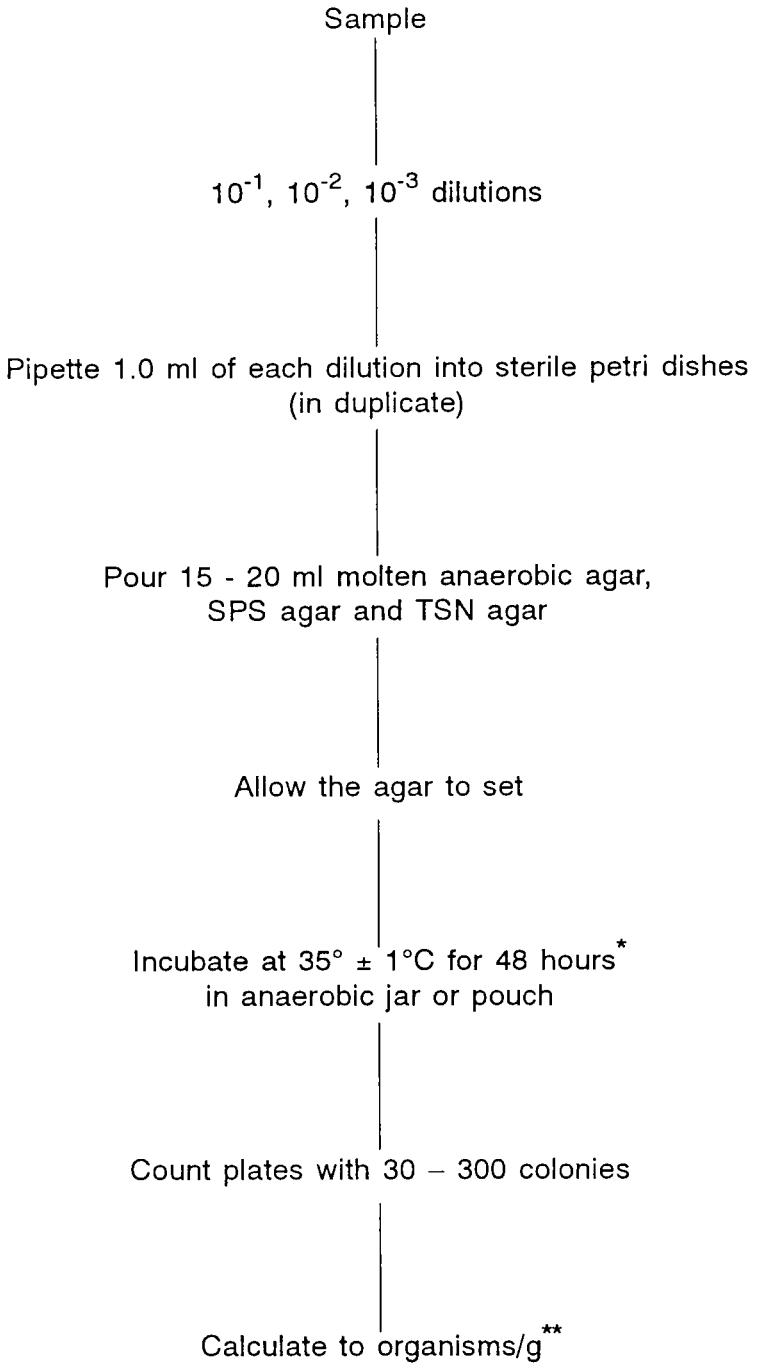
   Same as in aerobic plate count method, however culture media used are anaerobic agar (SPS agar and TSN agar).



* or at any selected temperature and specific period of incubation

** if only black colonies are counted, describe as anaerobic sulphite reducers per gram

Fig. 1. Flow diagram of the procedure for anaerobic plate count (Spread Plate Method)



* or at any selected temperature and specific period of incubation

** if only black colonies are counted, describe as anaerobic sulphite reducers per gram

Fig. 2. Flow diagram of the procedure for anaerobic plate count (Pour Plate Method)

REFERENCE

Shokuhim Eisei Kensa Shishin, Guide to Food Hygiene Examination (Authorized by the Ministry of Health and Welfare), Japan Hygiene Association (1990).