SPORE FORMING BACTERIA COUNT

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INTRODUCTION

Spores of bacilli could survive in mixed population of micro-organisms and could be found in foods commonly. These spores usually have greater resistance to temperature, germicidal chemicals and lethal radiations etc than the vegetative cells. Therefore, it is not surprising that spores are detected readily in many foods and ingredients such as starches, dried food stuff etc.

The following method uses heat shock to kill the vegetative cells and specific incubation temperature is selected to let the surviving spores to germinate for enumeration. However, the method described is not suitable for the enumeration of facultative and stenothermophilic organisms.

I. APPARATUS

Autoclave	Incubator
Weighing balance	Pipettes, 1 ml & 10 ml
'Waring' blender & flasks or Stomacher ('Lab-blender' 400)	Bunsen burner
Thermostatic controlled water bath with stirrer	Laminar flow chamber
Scissors, scalpel & forceps	500 ml Erlenmeyer flask
Alcohol (70% v/v) swabs	Sterile petri dish (90 mm ø x 15 mm H)
Colony Counter	

II. CULTURE MEDIA

1. Diluent: Select one of the following as diluent

   a. 0.1% Peptone Water

Peptone	1.0 g
Distilled water	1.0 liter

   Dissolve peptone in distilled water. Adjust to pH 7.0 ± 0.1. Dispense 45 ml each in glass bottle and sterilise at 121°C for 15 minutes.

   b. Butterfield's buffered phosphate diluent*

   *Refer to Appendix B.

c. 0.85% Saline Solution

Sodium chloride	8.5 g
Distilled water	1.0 liter

Dispense 45 ml each into flasks, bottles, or tubes and sterilise at 121°C for 15 minutes.

2. Tryptone glucose extract (TGE) agar (Difco) or Trypticase glucose agar (BBL)

Prepare 100 ml of Tryptone glucose extract (TGE) agar or trypticase glucose agar in a 500 ml Erlenmeyer flask and one additional flask of medium is used as a sterility control. Sterilise at 121°C moist heat for 15 mins and transfer to a 45°C water bath for cooling and let it remain in the water bath.

III. PROCEDURE

1. SAMPLING PROCEDURE

Randomly take 150-200 g of sample. Aseptically cut the sample into smaller portions and keep them in a sterile polyethylene bag or container. Store the cut sample in refrigerator (5°C) to maintain sample's integrity.

2. EXAMINATION PROCEDURE

i. Weigh 25 g of sample in a sterile blender jar and add 225 ml of sterile 0.1% peptone water into it. Blend the sample at high speed for 2 mins. If a stomacher is used, it requires 60 seconds.

ii. Pipette the blended sample into a set of 3 flasks of TGE agar which are held in the bath in the following sequence: 10 ml into the first, 1 ml into the second, and 0.1 ml into the third flask. Swirl the flasks gently to disperse the blended sample throughout the medium.

iii. Transfer the flasks without delay to a stirred water bath pre-adjusted to 80°C and heat for 30 minutes. (For fish paste product, temperature would be set at 100°C for 10 minutes.) The water level must be above the liquid level in the flasks. Agitate the flasks occasionally and gently to ensure the heat is evenly distributed.

iv. Transfer the flasks to a 45°C water bath after rapid cooling (cooling is done in cold tap water taking care that the temperature does not fall to the point where agar solidifies) and keep in the water bath for a period not more than 10 mins.

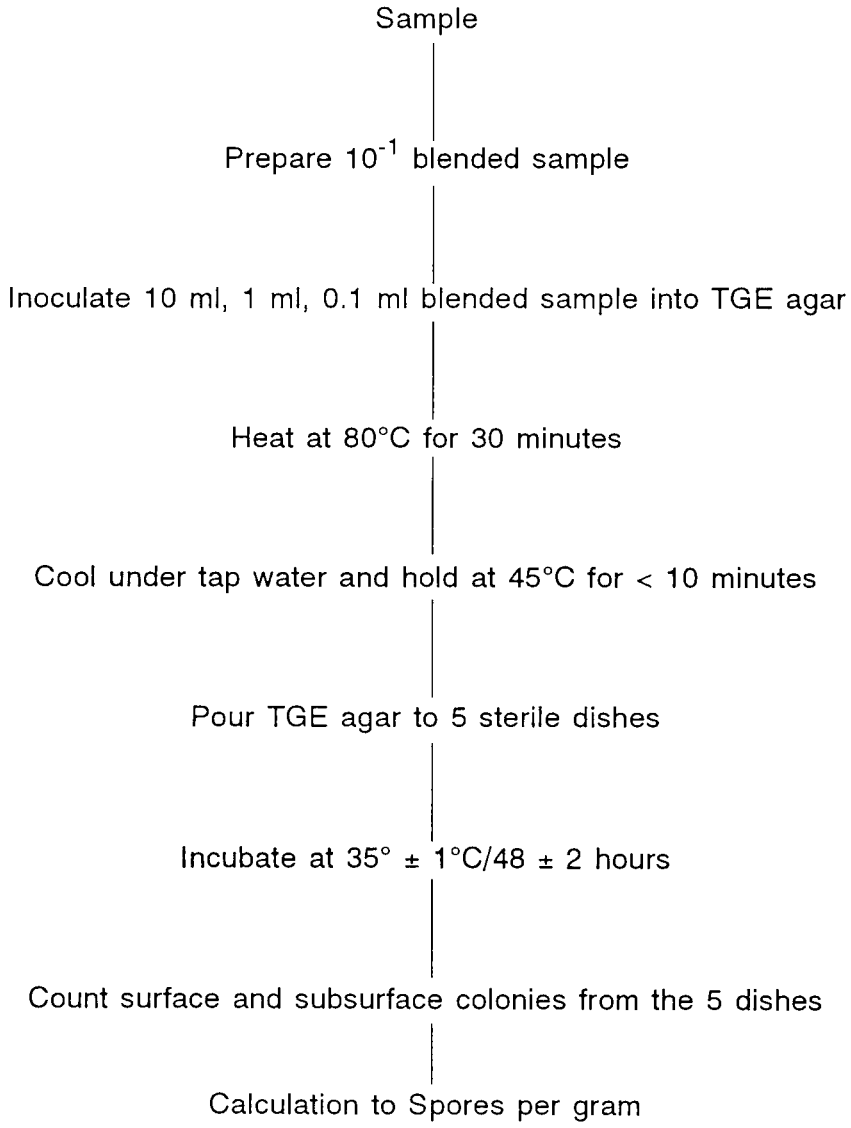
v. Pour the content in each flask, representing test sample and sterility control, respectively, into a set of 5 sterile petri dishes in equal volumes, i.e. about 20 ml per dish.

vi. When agar has solidified, invert and incubate the petri dishes at 35° ± 1°C for 48 ± 2 hours.

vii. Count the colonies on the surface and subsurface of the agar. The sum of colonies on the set of 5 plates poured from TGE agar containing 10 ml of blended sample, represents the number of aerobic, mesophilic spores per gram. Similarly, the number of colonies in sets of plates receiving 1.0 and 0.1 ml of blended sample is equal to 0.1 and 0.01 of the number of spores per gram and must be multiplied by 10 and by 100 respectively to get the count per gram.

viii. The number of spores which can be enumerated by this method ranges from 1 to 150,000 spores per gram.

ix. Flow diagram for aerobic spore-forming count:



REFERENCE

Compendium of Methods for the Microbiological Examination of Foods. Compiled by the APHA Technical Committee on Microbiological Methods for Foods. Edited by Marvin L Speck. 2nd edition. American Public Health Association, 1984.