AEROBIC PLATE COUNT

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INTRODUCTION

The aerobic plate count provides an estimate of the number of viable micro-organisms in food according to the medium used and the time and temperature of incubation. The spread plate and pour plate method described below is based on the assumption that each viable cell will form a colony, thus it is important that:-

- the sample is adequately dispersed.
- the cells do not multiply during the preparation of the dilutions.

The material under investigation is diluted in known volumes of sterile diluent to provide a set of serial dilutions of the microbial population so that an aliquot at some steps in the series provides 30 to 300 colonies when plated on a nutrient medium. (It is this range that will give the most accurate colony count.)

I. APPARATUS

'Waring' blender & flasks or
Stomacher ('Lab-blender' 400)

Pipettes, 1 & 5 ml

Scissors, scalpel & forceps

Alcohol (70% v/v) swabs

Bent glass spreader

Incubator

Colony Counter

Autoclave

Weighing balance

Laminar flow chamber

Bunsen burner

Sterile petri dish
(90 mm ø x 15 mm H)

II. CULTURE MEDIA

1. Plate count agar (PCA) or Standard Method agar

   Refer to respective manufacturer's instruction for the preparation of the medium.

2. Butterfield's buffered phosphate diluent

a. Stock solution:

Monopotassium hydrogen phosphate	34.0 g
Distilled water	500.0 ml

Adjust to pH 7.2 with about 175 ml 1N sodium hydroxide solution; dilute to one litre. Sterilise at 121°C for 15 minutes and store in refrigerator.

b. Working solution:

Dilute 1.25 ml stock solution to 1.0 litre with distilled water and dispense 45 ml each in glass bottles. Sterilise at 121°C for 15 minutes.

III. PROCEDURE

1. SAMPLING PROCEDURE

Randomly pick 150-200 g of sample. Aseptically cut each piece of the sample in half and keep the half-cut portions in a sterile polyethylene bag or sterile container. Store the bag/container in refrigerator (2° to 5° ± 1°C) to maintain sample's integrity and examine the sample within 18 hours. If test cannot be performed shortly, sample should be well frozen until further examination.

2. SAMPLE PREPARATION

i. Weigh aseptically 25 g of the above sample into a sterile 'Waring' blender jar or stomacher bag. Add 225 ml sterile Butterfield's buffered phosphate diluent and blend for 1 minute.

ii. Transfer 10 ml of the above suspension into 90 ml buffered phosphate diluent to give a dilution of 10^{-2}. Prepare next dilution (10^{-3}) by mixing 1 ml of the well mixed diluted sample solution (10^{-2}) with 9 ml buffered phosphate diluent. Prepare further dilutions: 10^{-4}, 10^{-5}, ... if required.

3. SPREAD PLATE METHOD

i. Select the appropriate dilutions from 2.ii above and for every dilution, inoculate 0.1 ml aliquot to each of two pre-dried PCA plates.

ii. Spread the inoculum gently and evenly over the surface of the agar plates with a sterile bent glass spreader.

iii. Allow the plates to stand until the inoculum has been absorbed completely, which should be within 15 minutes after the spreading.

iv. Invert the plates and incubate at 35° ± 1°C for 48 ± 2 hours or at selected temperature and period.

v. Count those plates which have 30-300 colonies.

vi. The aerobic plate count (Spread Plate Method) for the sample is calculated as follows:

APC = x.d/s colony forming unit(cfu)/g

where d : dilution at which colonies are counted.

x : average count of colonies.

s : volume of aliquot.

vii. Method of calculation:

Example:

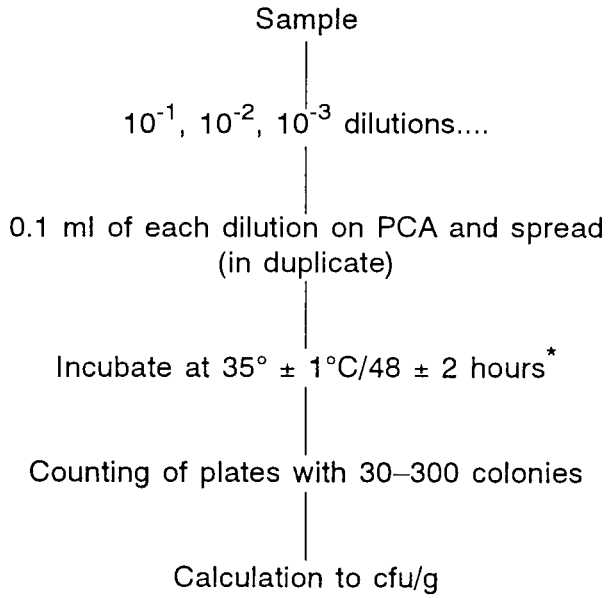
1. Colony count less than 30 per petri dish:

At dilution	Wt of sample/ml	Record result as
10^{-1}	0.1 g	< 300/g
10^{-2}	0.01 g	< 3,000/g
10^{-3}	0.001g	< 30,000/g

2. Colony count less than 30 and more than 300 per petri dish:

At dilution	Presumed average colony count from duplicate petri dish	Calculation
10^{-2}	2,854*	*Disregarded
10^{-3}	291	(291000+360000)/2 = 3.3 x 10^5/g
10^{-4}	36	
10^{-5}	4*	*Disregarded

viii. The procedure for Aerobic Plate Count (Spread Plate Method) is summarised as follows:



* or at selected temperature and period of incubation.

4. POUR PLATE METHOD

i. Select the appropriate dilutions from 2.ii above and for every dilution inoculate 1.0 ml aliquot each to a sterile petri dish.

ii. A 10-15 ml portion of molten PCA is poured into each of the above sterile petri dishes. The plates are then rotated 5 times clockwise, 5 times anti-clockwise 5 times back and forward. Care should be taken not to splash agar on the lid of the dish. Plates are left to set.

iii. Invert the plates and incubate at 35° ± 1°C for 48 ± 2 hours or at selected temperature and period.

iv. Count those plates which have 30-300 colonies on surface or subsurface of the agar.

v. The aerobic plate count (Pour Plate Method) for the sample is calculated as follows:

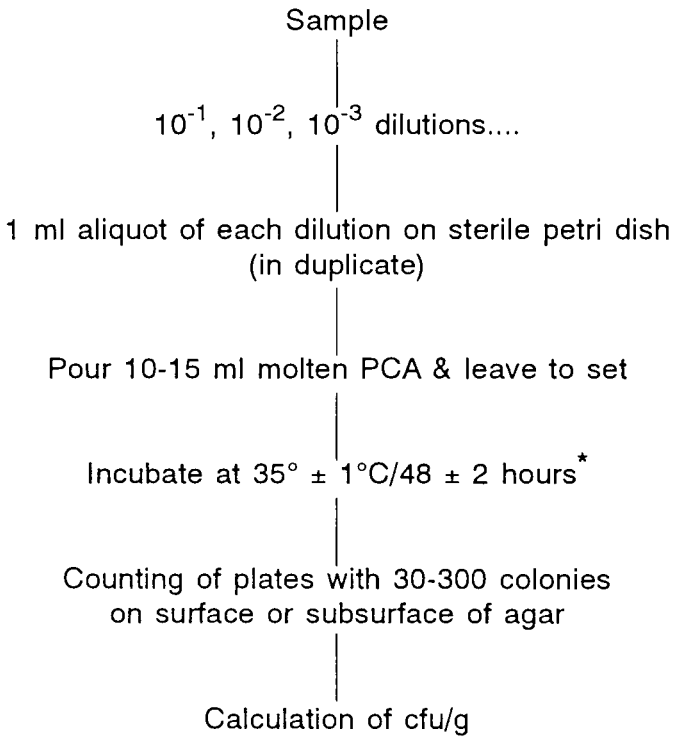
APC = x.d/s colony forming unit(cfu)/g

where d : dilution at which colonies are counted.

x : average count of colonies.

s : volume of aliquot.

vi. The procedure for Aerobic Plate Count (Pour Plate Method) is summarised as follows:



* or at selected temperature and period of incubation.

IV. REFERENCES

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2. Bacteriological Analytical Manual. Food and Drug Administration, Bureau of Foods, Division of Microbiology. 5th edition, August 1978.

3. 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.