

## Enumeration of total bacterial load in seafood by plate count method

Total plate count (TPC) or Aerobic plate count (APC) or Standard plate count (SPC) provides an estimate of the total number of aerobic microorganisms in foods including seafoods. It reflects on the general bacteriological quality of foods and is useful in determining the potential spoilage/deterioration of the perishable fish and fishery products, as an indicator of the sanitary conditions under which the food has been processed and/or produced and also of the level of Good Manufacturing Practices (GMPs) adopted during processing. It is enumerated by mixing a series of dilutions of the food homogenate with a non-selective agar medium and incubating at  $37 \pm 1^\circ\text{C}$  for 48 h. All the colonies developing on the medium are counted and bacterial load is expressed as colony forming units (CFU) per gram of sample. The total bacterial load in raw fish should not exceed  $10^5$  cfu/g.

### Materials required

1. **Diluent** : Phosphate buffer or Physiological saline (0.85%) or 0.1% Peptone water.
2. **Medium** : Nutrient Agar (NA) or Plate Count Agar (PCA) or Soyabean Casein Digest Agar (TSA). For marine bacteria, 1% to 3% NaCl is added and for salted fish, 5% NaCl is added.

### Procedure

About 25 g of fish muscle is weighed aseptically and homogenised with 225 ml physiological saline in a homogeniser / blender. Serial decimal dilutions of  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and others as appropriate depending on the type of sample are prepared with 9 ml physiological saline. 0.1 ml of inoculum from each of the serial dilution is poured onto Plate Count Agar (PCA) plates and spread using sterile glass spreaders for spread plate technique. But, in the case of pour plate technique, 1 ml of inoculum is placed into the petriplates and the sterile cooled ( $50^\circ\text{C}$ ) media is poured over it and mixed by gently swirling in clockwise and anticlockwise direction. The plates are incubated at  $37 \pm 1^\circ\text{C}$  for 48 h. All the colonies developed on the agar plates are then counted and counts per gram of sample calculated. It is advisable to choose dilutions which give colonies between 30 – 300 ranges. Plates having crowded or spreading colonies which cannot be counted shall be discarded.

### Calculation

#### *Pour Plate Technique*

Aerobic Plate Count (cfu/g) = (Number of colonies x Dilution) / Weight of sample

#### *Spread Plate Technique*

Aerobic Plate Count (CFU / g of sample) = (Number of colonies x Dilution x 10) / Weight of sample

### Media composition

#### *Nutrient Agar (NA)*

Peptone	-	5.0 g
NaCl	-	5.0 g
Beef Extract	-	1.5 g
Yeast Extract	-	1.5 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	$7.4 \pm 0.8$

Sterilize at  $121^\circ\text{C}$  for 15 minutes.

#### *Plate Count Agar (PCA) or Standard Methods Agar*

Tryptone	-	5.0 g
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Yeast Extract	-	2.5 g
Dextrose	-	1.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	7.0 ± 0.2

Sterilize at 121°C for 15 minutes.

***Soyabean Casein Digest Agar (Trypticase Soy Agar, TSA)***

Tryptone	-	17.0 g
Soya peptone	-	3.0 g
NaCl	-	5.0 g
Dipotassium phosphate	-	2.5g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	7.3 ± 0.2

Sterilize at 121°C for 15 minutes.

***Phosphate Buffer Solution***

***Stock solution***

Potassium dihydrogen orthophosphate (KH<sub>2</sub>PO<sub>4</sub>) - 34.0 g

Distilled water - 1000.0 ml

***Working solution***

Dilute 1.25 ml of stock buffer solution to 1000 ml with distilled water. Adjust the pH to 7.2 before use. Sterilize at 121°C for 15 minutes.

**Rules for enumerating counts**

1. Always choose replicate plates containing colonies between 30-300 and count all colony forming units (cfu) including those of pinpoint size. Record the volume and dilution used for plating, and average number of colonies counted.

Example:

Dilutions plated: 10<sup>-1</sup> 10<sup>-2</sup> 10<sup>-3</sup>

Colonies counted: > 300 80 20

> 300 70 10

Since only 10<sup>-2</sup> dilution shows colonies between 30 and 300, only this pair of plates is considered and average counts determined.

Average number of colonies x dilution factor

$$\text{CFU/ml} = \frac{\text{Average number of colonies} \times \text{dilution factor}}{\text{Volume of sample plated}}$$

Average number of colonies in 10<sup>-2</sup> = 80 + 70 ÷ 2 = 75

Volume of sample plated: 1 ml

CFU/ml = (75 x 10<sup>2</sup>) = 7.5 x 10<sup>3</sup>

Result reported as = 7.5 x 10<sup>3</sup> CFU/ML

2. If there are 2 consecutive dilutions showing between 30-300 colonies then the count in each of the dilutions computed. If the count of one dilution is not more than double the other, then the average of

both dilutions taken and results reported.

Example:

Counts in  $10^{-2}$  dilution : 280 and 290 colonies. CFU/ML =  $2.85 \times 10^4$

$10^{-3}$  dilution: 40 and 44 colonies. CFU/ML =  $4.30 \times 10^4$

Since 4.30 is not more than double of 2.85, the average is considered  
( $2.85 + 4.30$ )  $\div 2 = 3.47$  )

Result is report as :  $3.57 \times 10^4$  CFU/ML

3. If there are 2 consecutive dilutions showing between 30-300 colonies, and if the count of one dilution is more than double than the other then the lower value is reported.

Example:

Counts in  $10^{-2}$  dilution: 280 and 290 colonies. CFU/ML =  $2.85 \times 10^4$

$10^{-3}$  dilution: 80 and 70 colonies. CFU/ML =  $7.50 \times 10^4$

Since 7.50 is more than double of 2.85, the lower value is considered.

Result is reported as :  $2.85 \times 10^4$  CFU /ML

4. If there are no colonies in any of the dilutions, then the counts is reported as less than one times the lowest dilution plated.

Example:

$10^{-1}$  dilution: No colonies

$10^{-2}$  dilution: No colonies

Results reported as: **Estimated count**  $< 1 \times 10^1$  CFU/ML

or Est.  $< 1 \times 10^1$  CFU/ML

5. If all the dilutions show more than 300 colonies, then the dilution where the colonies are countable is considered. Then all the colonies in that dilution counted and the results computed as **Estimated count**.

Example:

$10^{-1}$  dilution : Too numerous colonies to count

$10^{-2}$  dilution: Too numerous to count

$10^{-3}$  dilution : 450 and 460 colonies (Average: 455 colonies)

Results reported as : **Estimated count**  $4.55 \times 10^5$  CFU/ML

6. If there are too numerous or too many colonies making the counting difficult, then results can be expressed by following one of the following ways.

Count all the colonies in 13 square centimeters using a colony counter and multiply the counts by 5. This number should be multiplied by the dilution factor used for plating to report as estimated count.

**OR**

Count all colonies in in 5 square centimeters using a colony counter and multiply by 13. This number should be multiplied by the dilution factor used to report the Estimated count.

**OR**

If the number of colonies is more than 100 per sq. cm, then count all colonies in one sq.cm and multiply it by 64. This number should be multiplied by the dilution factor to report the Estimated count.

*(Multiplying by 5 or 13 depends on the area of the petridish used. The standard petridish of diameter 9 cm has an area of approximately 65 sq. cm)*