

4. Aseptically spread the colony with the sterile glass spreader to the Muller Hinton plate
5. Allow the plate for dry 5 minutes
6. Place the antibiotic disc with the help of sterile forceps and gently press
7. Incubate the plate without inverting
8. After incubation measure the zone of inhibition.
9. Compare the measurement obtained from the individual antibiotics with the standard table to determine the sensitivity zone.

## **5. ENUMERATION AND ISOLATION OF PATHOGENS FROM SEAFOOD**

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## **Introduction**

Microbiology is the study of microorganisms like microscopic or barely visible single-celled life-forms such as bacteria, archaea, protozoans. Enumeration in microbiology is an estimation or determination of number of bacterial cells in a given sample. Enumeration of sea food has gained importance due to increased attention being paid to quality aspects of final product. The International Commission on Microbiological Specifications for Foods (ICMSF) established in 1962 to the need for internationally acceptable and authoritative decisions on microbiological limits for foods appropriate with public health safety, and particularly for foods in international commerce.

**Methods to enumerate microbes can be divided into two categories.**

- a) Total cell counts include dead and inactive cells.
- b) Viable methods only count cells that are metabolically active,

## **Direct Microscopic count/ Total cell count**

Direct microscopic counts measures number of cells in a population of a given sample under a microscope. This can be possible for liquid samples using special slides known as counting chambers, consisting of a ruled slide and a cover slip. It is constructed in such a manner that the cover slip, slide, and ruled lines delimit a known volume. The number of bacteria in a small known volume is directly counted microscopically and the number of bacteria in the larger original sample is determined by extrapolation. Bacteria can be counted easily and accurately with the petroff-Hausser counting chamber. This is a special slide accurately ruled into squares that are  $1/400 \text{ mm}^2$  in area; a glass cover slip rests  $1/50 \text{ mm}$  above the slide, so that the volume over a square is  $1/20,000 \text{ mm}^3$  i.e.  $1/20,000,000 \text{ cm}^3$ . If for example, an average of five bacteria is present in each ruled square, there is  $5 \times 20,000,000$  or 108, bacteria per milliliter.

## **Advantages:**

- a) It is quick way of estimating microbial cell number
- b) Morphology of the bacteria can be observed as they counted.

## **Limitations:**

- a) Dead cells cannot be distinguished from living ones. Only dense suspensions can be counted
- b) Difficulty in to count small cells
- c) Precision is difficult to achieve
- d) Require a phase- contrast microscope if sample is not stained.

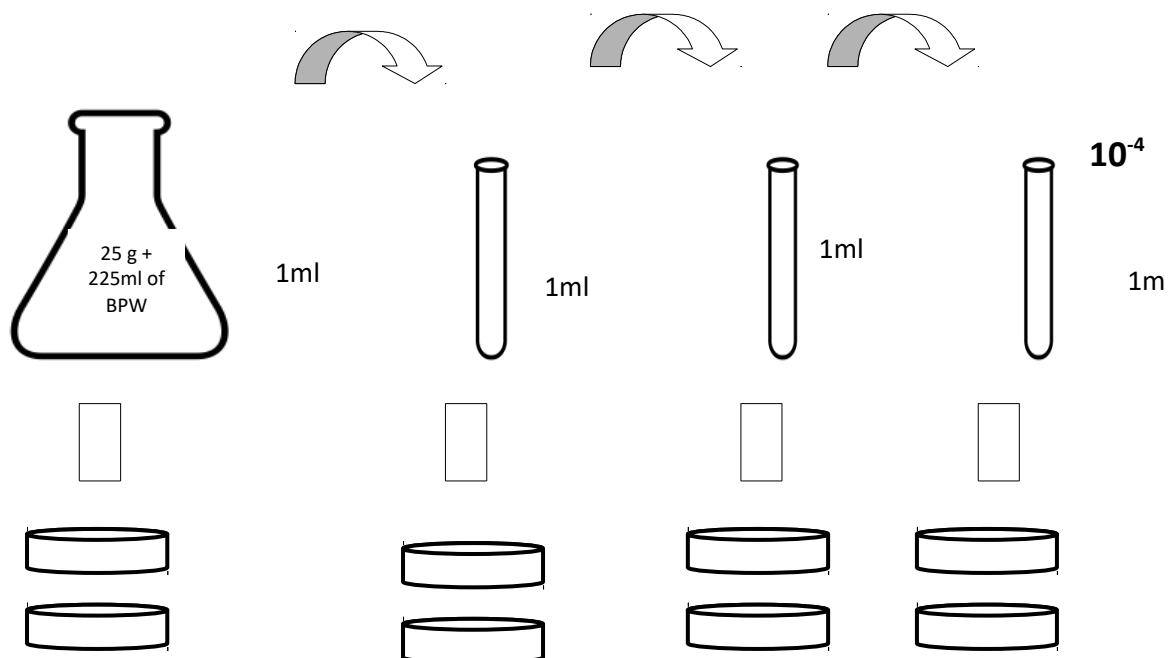
**Standard Plate Count (Viable Counts):** Any cell which has a capacity to divide and form a population or colony is defined as a viable cell. Viable count is also called as plate count or colony count. A viable cell count is usually done by diluting the original sample, plating aliquots of the dilutions on to an appropriate culture medium, then incubating the plates under suitable conditions for the colonies to be grown. Colonies are counted and, from a particular dilution used, the original number of viable cells can be calculated. For accurate determination of the total number of viable cells, it is critical that each colony comes from only one cell, so chains and clumps of cells must be broken apart. However, since one is never sure that all such groups have been broken apart, the total number of viable cells is usually reported as colony-forming units (CFUs) rather than cell numbers. This method of enumeration is relatively easy to perform but major disadvantage is the time necessary for dilutions, plantings and incubation.

**Total plate count (TPC):**

The Total plate count method is used for examining frozen, chilled, precooked, or prepared foods. It is also called as viable aerobic bacteria, total bacterial count, total plate count, total mesophilic count

- Take 25 g analytical unit fish to determine total plate count
- Add 225 ml of peptone salt solution or buffered peptone water - dilution to blender jar containing 25 g analytical unit and blend 2 min (This results in a dilution of  $10^{-1}$ ). Using separate sterile pipets, prepare decimal dilutions of  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and others as appropriate in BPW.
- Shake all dilutions 25 times in 30 cm (1 ft) arc within 7 s. and reshake dilution bottle 25 times in 30 cm arc within 7 s if it stands more than 3 min before it is pipetted into petri dish.
- Pipete 1 ml of each dilution into separate, duplicate, appropriately marked petri dishes.
- Add 12-15 ml plate count agar (cooled to  $45 \pm 1^{\circ}\text{C}$ ) to each plate within 15 min of original dilution.
- After solidification of agar, Invert the solidified petri dishes, and incubate promptly for  $72 \pm 2$  h at  $30^{\circ}\text{C}$ . Do not stack plates when pouring agar or when agar is solidifying.
- Select plates with 25-300 colonies including those of pinpoint size

**Flowchart -Isolation of Total Plate count (TPC)**



25 g of fish sample + 225 ml of sterile Buffer Peptone Water (BPW)



Macerate in a blender



Prepare tenfold dilution (10-1, 10-2, 10-3, 10-4) in BPW tubes (9ml)



Add 1ml of sample of each dilution into sterile petri dishes (in duplicates) Pour 15-20ml of Plate Count Agar (PCA) into each petridish



Invert the plates and incubate at 30 °C for 72 hrs



