Determination of number of microorganisms in Food Sample

It is an important point in food microbiology to indicate the level of food contamination or food pollution. There are several different ways for determining of M.O. (Bacterial growth may be determined by using either **Cell Mass methods** or **Cell Number methods)** in any sample and the most important methods are:

1. Cell Number methods:

- Direct Microscopic Counts (hemocytometer)
- Plate count method
- Most Probable Number
- Membrane Filter paper

Enumeration methods may yield either total counts or viable counts. Total count is a count of cells including dead and live cells. While viable count is a count of only those cells that are alive in the sample.

2. Cell Mass methods:

- Turbidity measurement by Spectrophotometry
- Metabolic assay
- Dry weight determination.

1. Direct Microscopic Counts (hemocytometer)

Direct microscopic counts of microbial cells may be counted directly by observing the total number of cells. This method is used in food microbiology to determine the sanitation of products e.g milk.

Advantages:

- Rapid Method.
- It will count the bacteria that don't form colony on agar plate at $35^{o}C$ (Thermophilic & Psychrophilic).

- In case of milk presence of excess number leukocyte and pus cell will be evidence that the animal is infected by mastitis.

Disadvantage: They don't discriminate between living and death cells

Procedure:

1. Clean Petroff-Hausser (P-H) counting chamber with 70% alcohol and let air dry.

2. Make **1:10** dilutions as instructed, based on sample from 10^{-1} - 10^{-6}.

3. Dispense **10 µl** of diluted bacterial suspension on counting chamber (between the edge and cover slip. Let it settle down for **5 minutes**.

4. After **5 minutes** put the hemocytometer under high power (40X objective lens) and focus to observe the grid formed on the hemocytometer. You have to count the bacterial cells in the central square that is itself divided into **25 squares**.

5. When you count is complete then continues with the calculations in following way:

a. For example, you counted 450 cells in 5 squares.

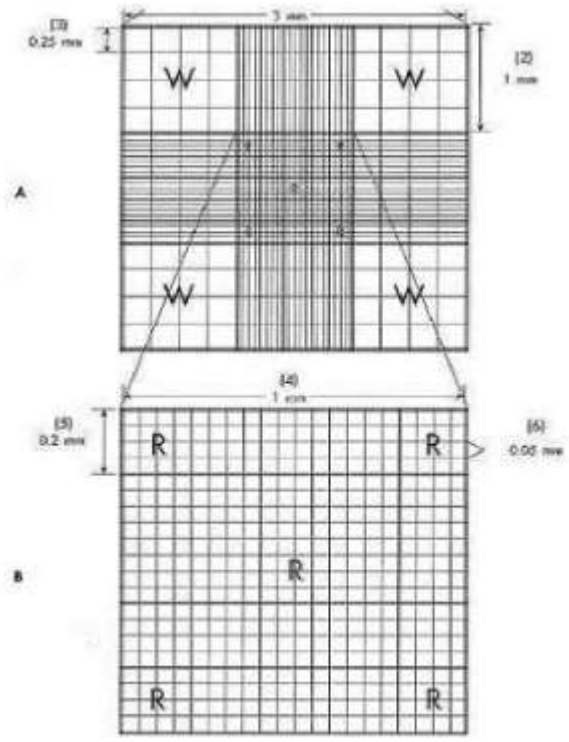
b. We know that area of 1 big central square is 1 mm^2, since central square has 25 small square so that area of 1 small square is 1/25 = **0.04 mm^2**. As you counted 5 squares, area of 5 squares is 0.04 x 5 = **0.2 mm^2.**

c. We also know that depth of this grid chamber is **0.1** mm. So that volume of the chamber is area x depth. It is 0.2 x 0.1 mm = **0.02 mm^3**.

d. No of cells per 1 mm^3 will be $450/0.02 \times \frac{1}{\text{dilution factor}}$

e. If you used dilution of 10^{-3}, for example, then it will be 450/0.02 x 1000

f. 22500000 = 2.25 x 10^7 cells per ml



2. Plate Count Method:

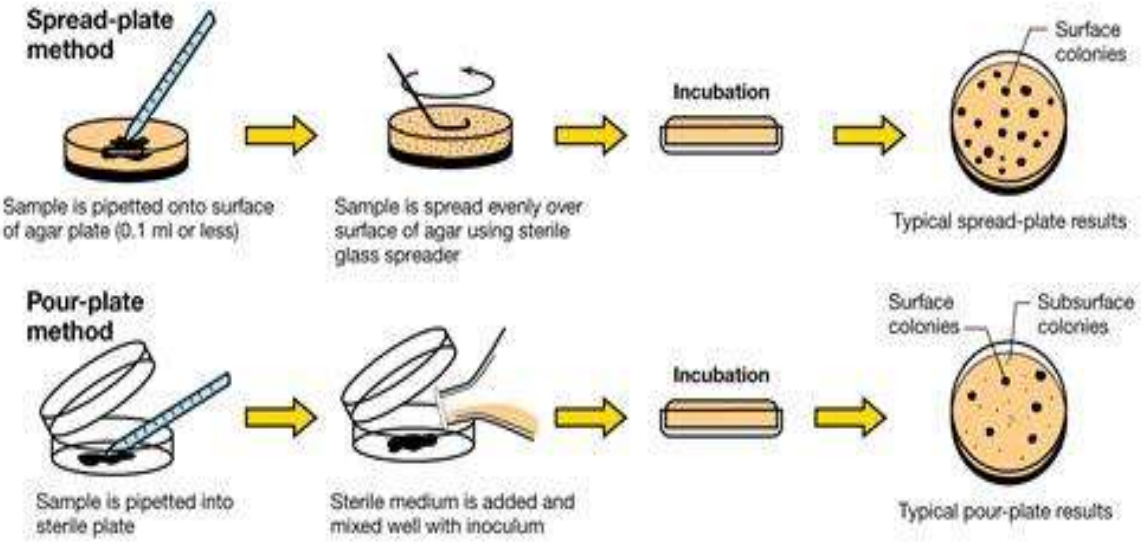
***This is the most commonly used method for enumeration of bacteria in a wide variety of samples including milk, food, meat, soil etc.

***Time consuming but technically simple method that does not require sophisticated equipment. Generally used only if the sample has at least 10^2 cells/ml. There are two types:

a. Pour Plate Method (PPM).

b. Spread Plate Method (Spot Plate Method) (SPM).

In pour plate method (PPM) the media will be added after putting **1ml** of sample into the Petridis, while in **spread plate method (SPM)** the media will be added into the Petridis then **0.1ml** of sample is spreaded entirely on the surface of the media.



Bacterial Count of a Food Product (meat)

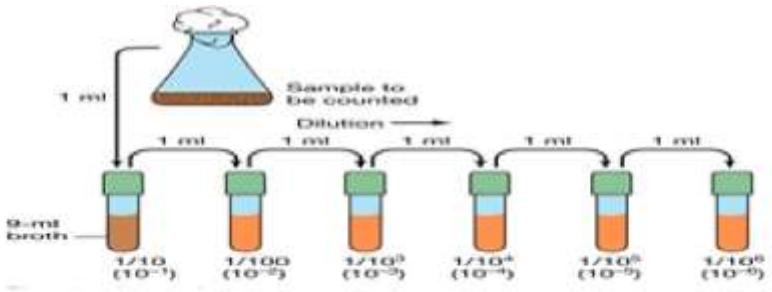
Aim:

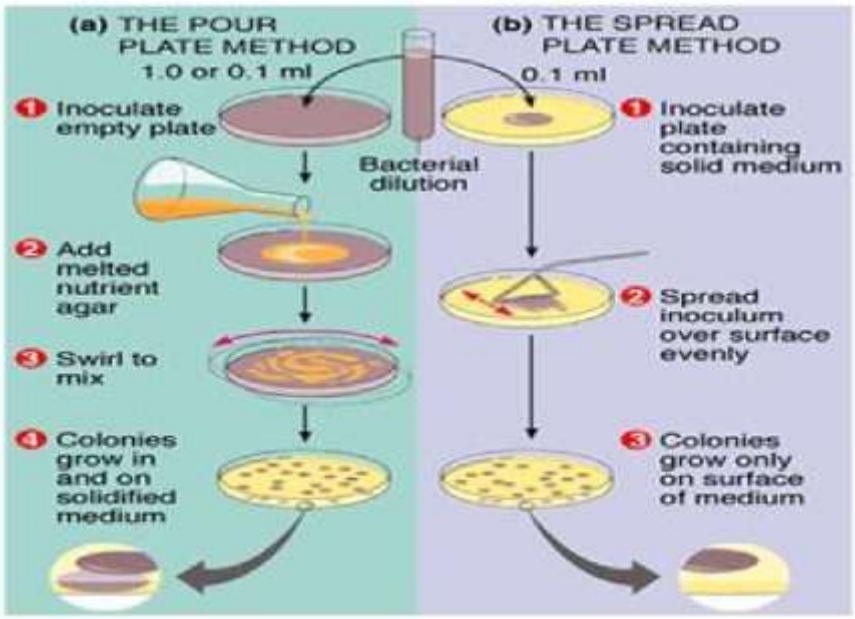
Determine the number of bacteria in different types of meat samples by performing a standard plate count

Procedure:

1. Weight out 10 g of raw hamburger or chicken.
2. Blend the 10 g of the meat in a blender with 90 ml of sterile distilled water for 5 minutes. This gives a 1/10 (10^{-1}) dilution.
3. Make dilutions from 10^{-1} to 10^{-6} as indicated in below figure.
4. Shake the 10^{-4} dilution again and aseptically transfer 1ml volume of dilutions that are to be plated onto empty sterile Petri plate and add nutrient agar (Melt nutrient agar in a water bath at temperature 48C° to 50°C and cools). Gently swirl on a flat surface in a figure-eight motion and allow hardening(PPM).
5. Add 0.1 ml of the 10^{-4} dilution to nutrient agar plate for spreading(SPM)

6. Using aseptic technique, pipette aliquots from the dilution blanks to the petri plates.
7. Incubate the plates in an inverted position for 24 to 48 hours at 35°C.





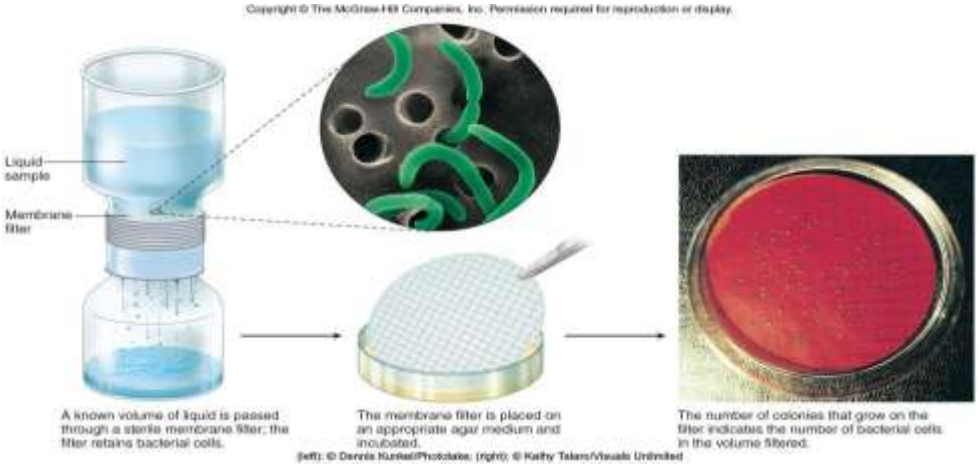
8. Use the formula:

In PPM, No. of M.O. in 1ml = No. of colony x $\frac{1}{d.f}$

In SPM, No. of M.O. in 1ml = No. of colony x $\frac{1}{d.f}$ **x 10**

3-Membrane filtration paper

Used for samples with low microbial concentration. A measured volume (usually 1 to 100 ml) of sample is filtered through a membrane filter (typically with a 0.45 μm pore size). The filter is placed on a nutrient agar medium and incubated. Colonies grow on the filter and can be counted.



4-Most probable number (MPN):

Most Probable Number (MPN) & Membrane Filter methods usually are used to enumerate the numbers of bacteria in water samples.

The **Most Probable Number** method is used to check **potability** (if water is safe enough to be drinking water) of water.

The MPN method looks for the presence of potential pathogenic bacteria that may be in the water due to **fecal contamination** of the water supply.

Water supplies are generally derived from ground sources and have to be checked for safety levels of bacterial contamination.

MPN method enumerates the enteric bacteria called coliforms, specifically **fecal coliforms** (*E. coli*)

Coliforms are **Gram negative bacilli that have the ability to ferment lactose with the production of acid and gas**.

Fecal coliforms are those coliforms that are normally found in the feces of warm blooded animals (including humans)

MPN method thus enumerates the **fecal coliforms** in water samples.

E. coli is thus used as an **indicator organism**.

MPN test includes 3 levels of testing:

- **Presumptive.**
- **Confirmed.**
- **Completed.**

The **presumptive** test looks for presence of fecal coliforms in the water sample by inoculating lactose broths with the water sample.

Those tubes that show presence of **acid and gas** are scored as positive test and those with **no acid/gas** as negative**.**

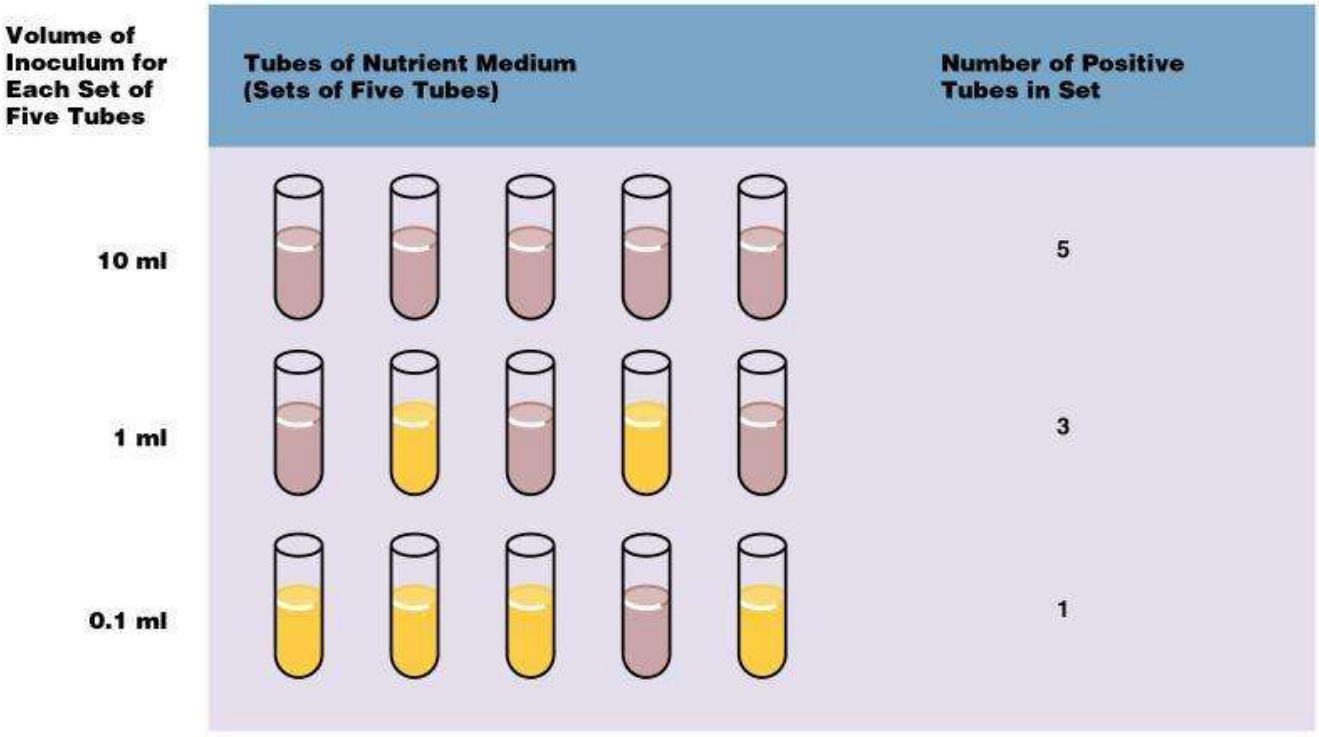
Three sets of lactose broths are inoculated with varying dilutions of the sample; First set of 3 or 5 tubes inoculated with 10 ml of sample; Second set of tubes inoculated with 1 ml of sample and third set of tubes inoculated with 0.1 ml of sample.

The combination of positives in the 3 sets is used to figure out the MPN /100 ml of water using the table provided.

The tubes that show positive in the **presumptive** test should be confirmed to contain *E.coli.*

This done in the **confirmed** test using the selective (differential) medium **EMB** (that uniquely highlights *E.coli* growth on it).

The **completed** test is done only where legal issues are involved wherein the bacterial culture is then identified by a full complement of tests including gram stain to show that it is indeed *E.coli*



(a) Most probable number (MPN) dilution series

Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	9	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9	86
5-0-1	30	10	110
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360

(b) MPN table

Cell Mass methods

1-Turbidimetric Method:

In this type, the procedure depends on the absorbance of photons by cells inside the sample and the spectrophotometer is used for such purpose on 590 or 600 nm.

Quick and efficient method of estimating the number of bacteria in a liquid medium is to measure the turbidity or cloudiness of a culture and translate this measurement into **cell numbers**. This method of enumeration is fast and is usually preferred when a large number of cultures are to be counted.