

Aerobic Mesophilic Plate count (APC) OR
Total Bacterial Count (TBC) OR
Total Aerobic Viable Bacteria (TAVB)

Indicates microbial counts for quality assessment of foods

Equipment:

Refer to lab1 (Equipment, Materials & Glassware).

Medium:

- Plate Count Agar (**PCA**)
- Nutrient Agar (**NA**)
- Peptone water 0.1%.

Procedure:

Preparation of food homogenate

Make a 1:10 dilution of the well mixed sample, by aseptically transferring sample to the desired volume of diluent.

1-Measure **non-viscous liquid samples** (i.e., viscosity not greater than milk) volumetrically and mix thoroughly with the appropriate volume of diluent (**1ml into 9ml, or 11 ml into 99 ml, or 10 ml into 90 ml or 50ml into 450 ml**).

2-Weigh **viscous liquid sample** and mix thoroughly with the appropriate volume of diluent ($11 \pm 0.1\text{g}$ into 99ml; $10 \pm 0.1\text{g}$ into 90ml or $50 \pm 0.1\text{g}$ into 450ml).

3-Weigh $50 \pm 0.1\text{g}$ of **solid or semi-solid sample** into a sterile blender jar or into a stomacher bag. Add 450 ml of diluent. Blend for 2 minutes at low speed (approximately 8000 rpm) or mix in the stomacher for 30-60 seconds.

4-**Powdered samples** may be weighed and directly mixed with

the diluent.

Shake vigorously (50 times through 30 cm arc).

In most of the food samples particulate matter floats in the dilution water. In such cases allow the particles to settle for two to three minutes and then draw the diluent from that portion of dilution where food particles are minimum and proceed.

Dilution:

If the count is expected to be more than 2.5×10^6 per ml or g, prepare decimal dilutions as follows. Shake each dilution 25 times in 30 cm arc. For each dilution use fresh sterile pipette. Alternately use auto pipette. Pipette 1 ml of food homogenate into a tube containing 9 ml of the diluent. From the first dilution transfer 1ml to second dilution tube containing 9ml of the diluent.

Repeat using a third, fourth or more tubes until the desired dilution is obtained.

1-The standard plate count (SPC) , (Pour plating)

Pour plating:

Label all petriplates with the sample number, dilution, date and any other desired information. Pipette 1ml of the food homogenate and of such dilutions which have been selected for plating into a petri dish in duplicate.

Pour into each petri dish 10 to 12ml of the molten PCA (cooled to 42-45 C) within 15 min from the time of preparation of original dilution. Mix the media and dilutions by swirling gently clockwise, anti-clockwise, to and fro thrice and taking care that the contents do not touch the lid. Allow to set.

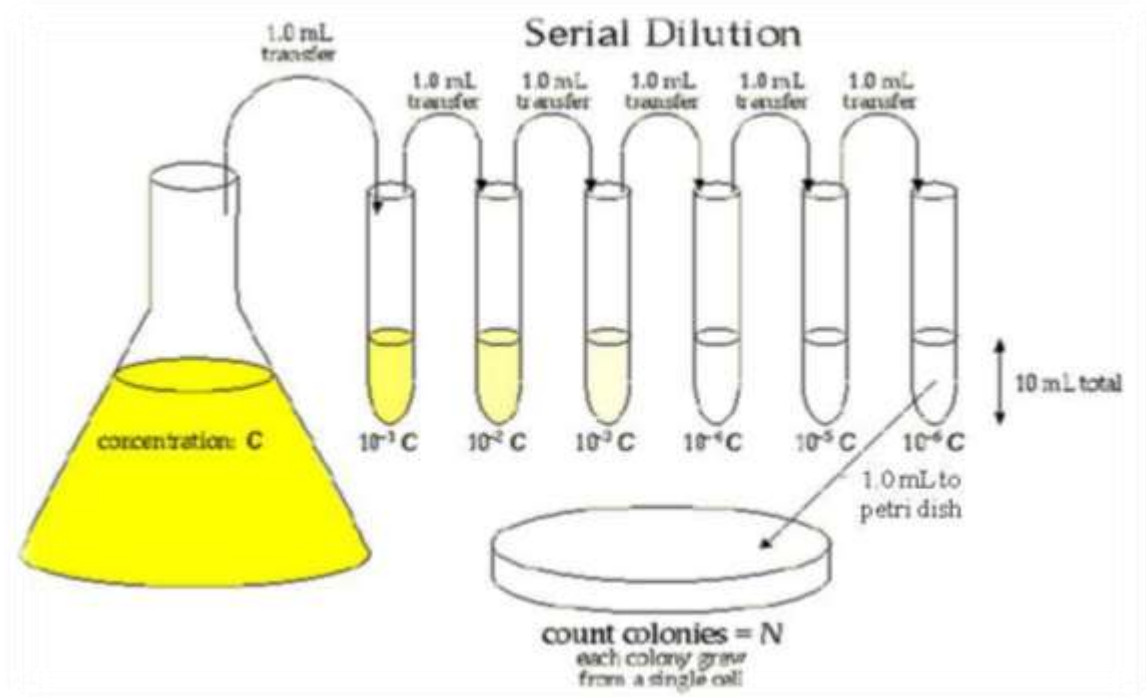


Figure: Serial dilution of samples in tubes containing 9ml of sterile distal water .

Incubation:

Incubate the prepared dishes, inverted at 35 C for 48±2 hours. (Or the desired temperature as per food regulation e.g. in case of packaged drinking water).

Counting Colonies:

Following incubation count all colonies on dishes containing 30-300 colonies and record the results per dilution counted.

Calculation

In dishes which contain 30-300 colonies count the actual number in both plates of a dilution and as per the formula given below:

Expression of Result

Aerobic (Mesophilic) Plate Count = 19000 CFU/g or 1.9×10^4 CFU/g