EQUIPMENT, MATERIALS ANDGLASSWARES

Food Sampling and Preparation of Sample Homogenate

A. Sampling plans

B. Equipment and materials

C. Receipt of samples

D. Thawing

E. Mixing

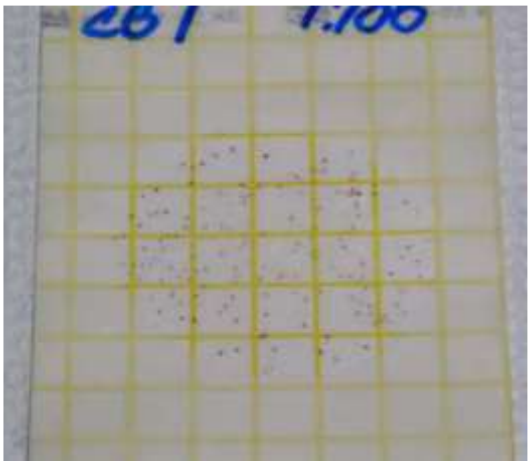
F. Weighing

G. Blending and diluting of samples requiring enumeration of microorganisms

A. Sampling plans :

The tests are determined according to the food type from this list of tests:

1. **TBC:** Total Bacterial Count ,Aerobic Plate Counts .



Aerobic Count

Count x Dilution Factor is reported

2.TCC :Total Coliform Count and fecal coliform , *Escherichia coli* (including enteropathogenic strains) .

3.TYMC: Total Yeast Mold Count .

Coliform counts, Aerobic counts, Mold & Yeast counts give an indication of the general cleanliness of food.

4. TPBC: Total Psychrophilic Bacterial Count.

5. TTBC: Total Thermophilic Bacterial Count.

6. TSC: Total Spore Count.

7.Tests for pathogenic bacteria

Salmonella species ,*Staphylococcus* spp., *Vibrio* spp., *Shigella* spp., *Campylobacter* spp., *Yersinia* spp., *Bacilluscereus*, and *Clostridium perfringens* .

8. Tests for parasites.

9. Tests for viruses.

10. Tests for mycotoxins .

B. Equipment and materials :

1- Mechanical blender. Several types are available. Use blender that has several operating speeds or rheostat. The term "high-speed blender" designates mixer with 4 canted, sharp-edge, stainless steel blades rotating at bottom of 4 lobe jar at

10,000-12,000 rpm or with equivalent shearing action. Suspended solids are reduced to fine pulp by action of blades and by lobular container, which swirls suspended solids into blades. Waring blender, or equivalent, meets these requirements.

2- Sterile glass or metal high-speed blender jar, 1000 ml, with cover,

resistant to autoclaving for 60 min at 121°C

3- Balance, with weights; 2000 g capacity, sensitivity of 0.1 g

4- Sterile beakers, 250 ml, low-form, covered with aluminum foil

5- Sterile graduated pipets, 1.0 and 10.0 ml

6-Butterfield's phosphate-buffered dilution water, sterilized in bottles to yield final volume of 90 ± 1 ml

7- Sterile knives, forks, spatulas, forceps, scissors, tablespoons, and tongue depressors (for sample handling)

MPN: Most probable number method, used to estimate the number of cells of a particular class of organisms in a food sample, when that number is expected to be too low to be detectable by direct plating methods.

Standard: Part of a law or regulation that is enforceable by a particular regulatory agency. In the case of microbial standards, it is the limit of the number of organisms, or of positive samples, allowable by that agency.

Sterilization: Process by which foods are exposed to levels of heat that are high enough to destroy all viable microbial cells.

Pasteurization: Process by which foods are exposed to levels of heat that are high enough to destroy all disease-producing vegetative cells, or to reduce the number of spoilage organisms.

Methods of Counting Bacteria

There are two standard methods of counting bacteria:

1-**The Standard Plate Count (SPC) or Pour Plating** and

2- The Most Probable Number (MPN)

3-Direct Microscopical Count (DMC)

methods. Either technique can be used with selective or nonselective technique – the method of choice depends largely upon the number of bacteria to be counted in the sample. The SPC is routinely used for samples that have a relatively large number of bacteria, which can be diluted down and grown as a countable number of colonies in a Petri plate.

The MPN method is used when the number of bacteria to be counted is so low that the cells could not be detected if a sample were applied to a plate, which is often the case when counting coliform bacteria.

I. Standard Plate Count of Bacteria in Food Products

The **Standard Plate Count** is the most common method used to quantify bacteria in foods.

To perform a standard plate count, the food to be tested is suspended in liquid and a sample is then spread over the surface of a solid medium in a petri plate. Bacterial cells present will form colonies that can be counted to determine the number of cells in the original sample. When the objective is to estimate the total number of bacteria, a complex medium called **Plate Count Agar** is commonly used since it will support growth of many different types of bacteria. We call the results the number of **Colony Forming Units (CFU)**, not total bacteria. This is because no single culture medium will support all different types of bacteria, we can only count those that do grow to form a visible colony.

Serial Dilution of samples

When performing a bacteria count, **between 30 and 300 bacterial colonies** need to be on the plate. A minimum of 30 assures that the data is statistically reliable; however, if there are more than 300 colonies are present, competition for nutrients can suppress growth of colonies.

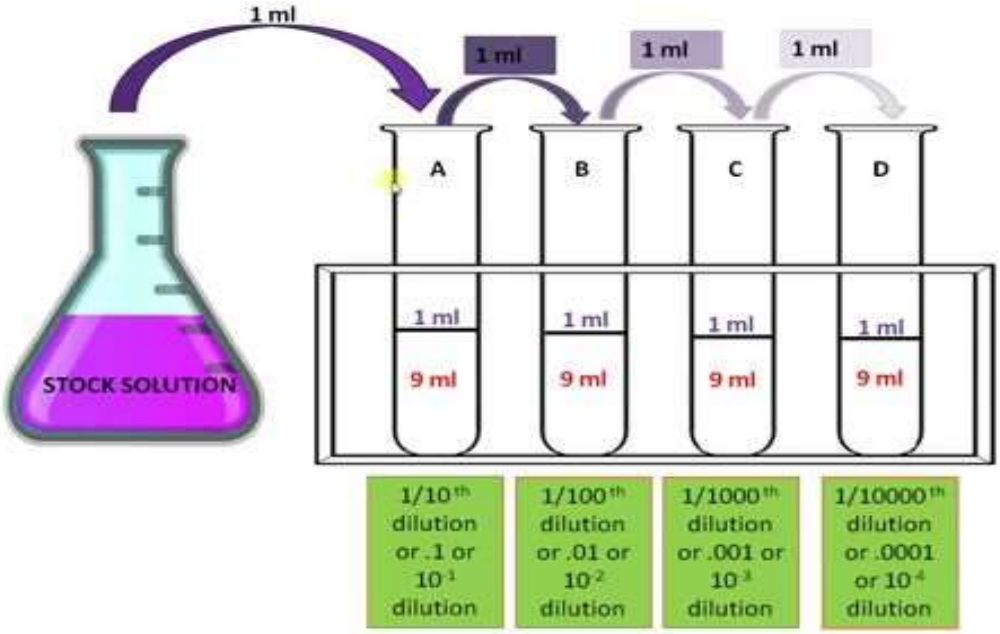


Figure. Example dilution series for a sample.

Your dilution series will be longer, and you will plate the 4 highest dilutions

2. Using the Most Probable Number Technique to Count Coliform in Water . MPN: Most probable number method, used to estimate the number of cells of a particular class of organisms in a food sample, when that number is expected to be too low to be detectable by direct plating methods.

In water, only a few coliform per liter can represent a potential health hazard. In this situation, the concentration of cells is too low to count

with a standard plate count (no cells may be present within any particular 1 ml sample).

3-Direct Microscopical Count (DMC)

The DMC method is used to count bacteria in liquid foods by the Microscope i.e., milk , and this method is different in other methods to be count all the death and live bacterial cells present of samples .