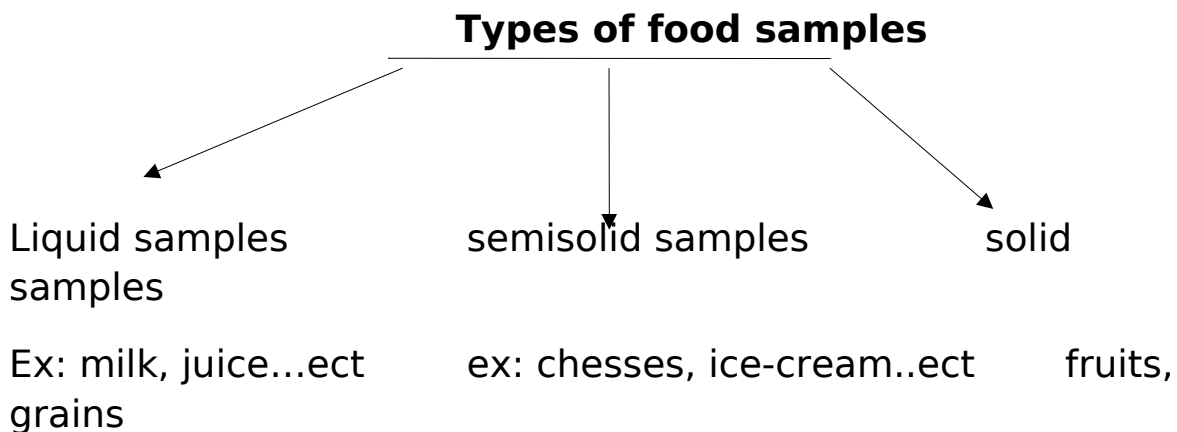


**Food microbiology:** is the study of the spoilage & pathogenic microorganisms that inhabit in food, mainly Accompanied by changes in the food.



- In liquid sample shake before sampling for homogenization.
- In solid sample the sampling done by using sterile knife or cork borer.
- Some samples done by taking thin layers from the surface.

### Dealing procedures with the sample in lab

#### **Sampling (food homogenate):**

It is about 10 gm or ml is collected from food.

#### The mortar

Mash or crush the solid foods and turn them into emulsion.

#### The container

---

Sterile, wide-mouth, glass or plastic are used.

### Instruments

Used Probe (trial) spoon & knife to cutting & transport sample.

### Sampling report

- 1- Date of sampling.
- 2- Nature of food.
- 3- Suggested tests.
- 4- Any useful information.

### **Preparation & dilution of food homogenate:**

Aseptically, 10 gm are transferred into sterile container, 90 ml diluted and shaken several times by mortar to obtain a  $10^{-1}$ . the mixture is left for 3-5 min just before making dilution.

### **Dilutions**

The food homogenate is mixed & serial ten -fold dilutions are made.

Tubes containing 9ml volume of diluents are prepared in a row.

These are numbered in order with the ten-fold dilutions ( $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , ect).

1ml of the  $10^{-1}$  dilution is transferred into the first of the 9 ml tubes. each 1 ml transferring from the previous tube into next.

## Media

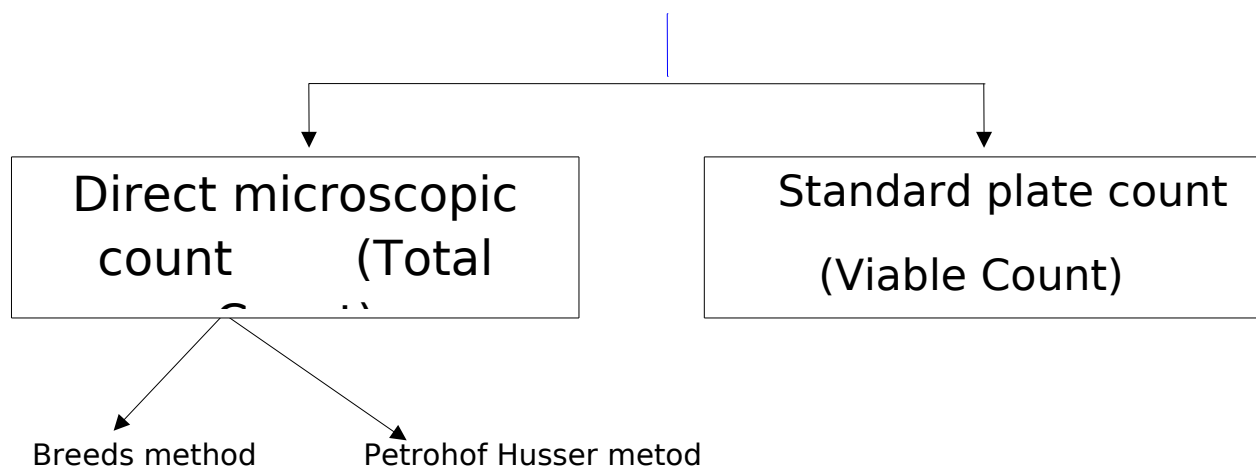
Pour plating

1 ml of each ten-fold dilution is put in Petri dishes about 15-20 ml of the molten agar & allowed to solidify.

## Incubation

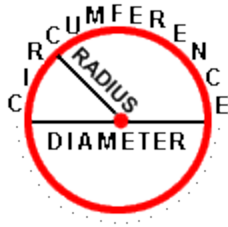
The plates are incubated in an inverted position for 24-48 hours at 37C°.

## Determination of M.Os Numbers



## Breed method:

This method is used to count the number of microbe cells (live and dead) is characterized as easy and the speed



Area for Circular microscopic field =  $\pi = 3.14$ .

Diameter microscopic field =  $160 \gg \gg 80 * 80 * 3.14 = 20096$

To prepare area of the bacterial film, draw a square 1 cm on slide.

Transfer 0.01 ml or drop by loop to slide and spread, wait to dry.

By Methylene blue dye for then washed and examines

Calculate the number of microbes cells then take the rate of 10 fields.

$1\text{cm}^2 = 100\ 000\ 000$  Micron

Area of the drawn square

Number of microscopic fields in  $1\text{cm}^2 =$  \_\_\_\_\_

One Area of the microscopic field

100000000

=  $5000 \sim 4976 =$  \_\_\_\_\_ = Microscopic coefficient

20096

Loopfull = 100

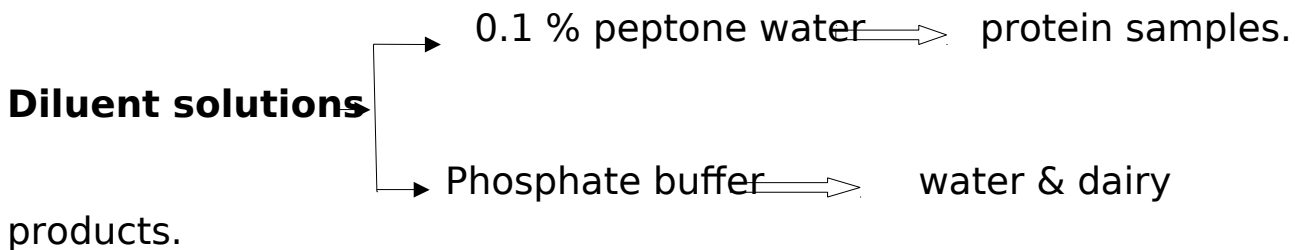
Number of microbial cells in 1 ml = coefficient microscopic \*  
average number of cells \* invert of dilution \* drop volume

**Q/Calculate the number of microbial cells in half a liter of milk if you know that the loopfull from second dilution and the average number of cells 25 cells?**

### **Standard plate count (Aerobic plate count)**

Standard plate count is designed to determine viable bacterial density in food or water sample.

Standard plate count is based on mixing decimal dilutions of food sample .after incubation of plates at 37C° for 24-48 hrs , the NO. of bacteria per ml is calculated from the NO. of colonies obtained in selected petri dishes at levels of dilutions giving significant results.



### **Dilutions:**

The food homogenate is mixed or bottle should be shaken, serial decimal dilutions (tenfold) are made .for example  $10^{-2}$  &  $10^{-3}$  etc dilutions.

**Pour plating & incubation**

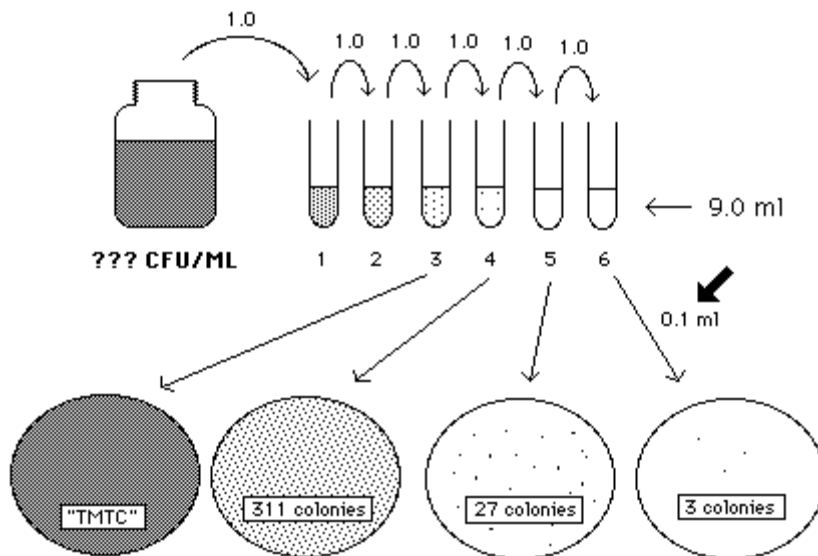
- One ml of each ten-fold dilution is added into duplicate plates.
- About 15-20 ml of the molten plate count agar (44-46C°) are added to each of the duplicate within 15 minutes & allowed to solidify.
- The plats are incubated for 24-48 hrs at 37 C°.

**Counting & calculation of colony - forming units (CFU):**

Only the plates containing 30-300 CFUs are counted.

When the counting the NO. of bacteria per gm or ml , the total count is calculated as follows:

Colony forming unit (CFU) = invert of dilution factor \* No. of colonies



TNTC= too many to count >>> more than 300 colonies

TFTC= to few to count >>> less than 30 colonies

**LAB.Method**

Food samples (10g or 10 ml) + 90 ml → 1<sup>st</sup>. Dilution

Highly contamination sample  
Sample

low contam.

(serial dilutions ... 10<sup>-x</sup>)

(1<sup>st</sup> - 2<sup>nd</sup> dilution)

Pour plate method

(put inoculum 1ml or 0.5 ml on sterile petri dish under sterile conditions)

The medium (general or selective)

Incubation at 37c for 24 hrs

Microbial count

(Direct plate count or standard plate count )

Identification

Large

Large Colonies

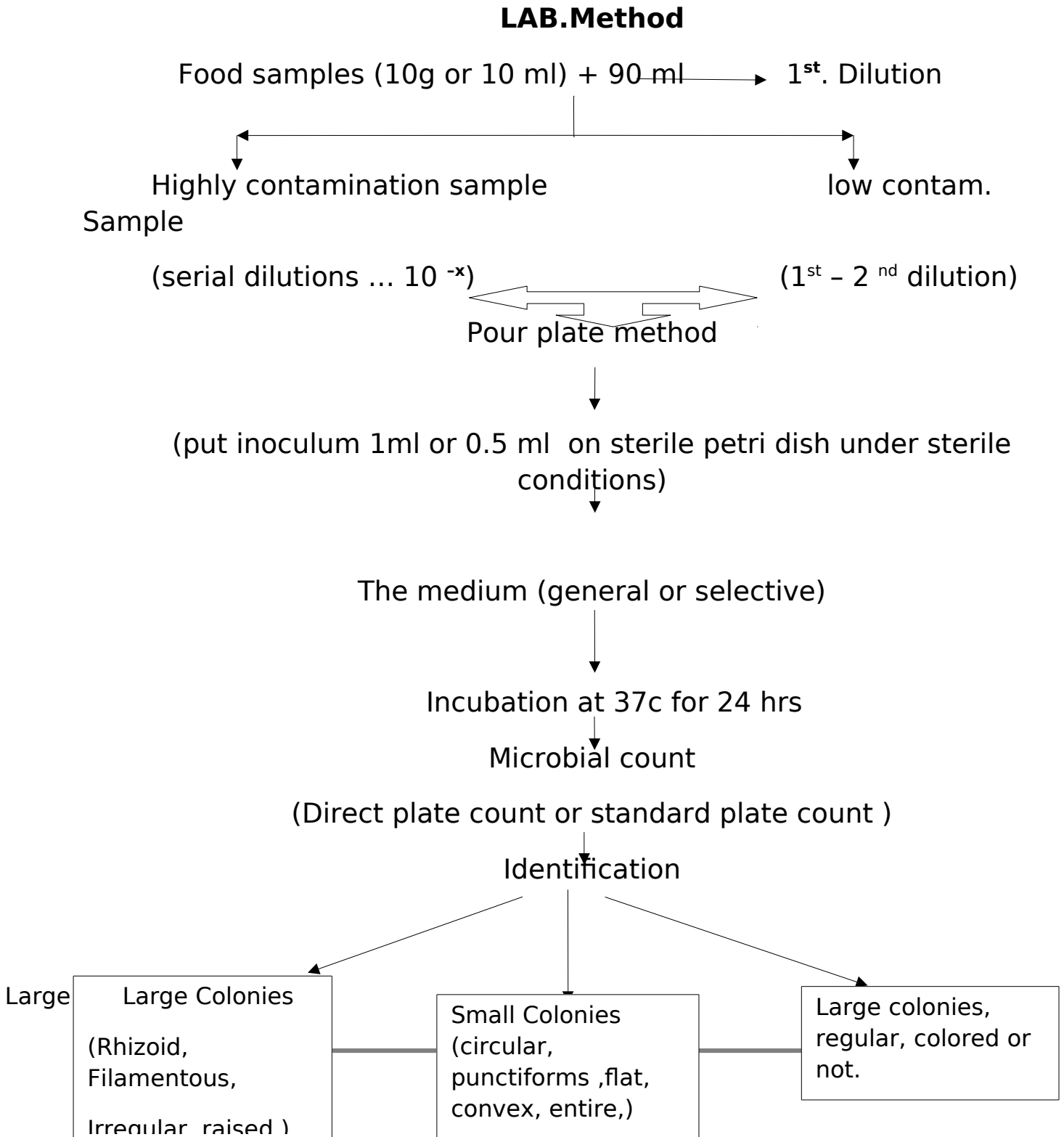
(Rhizoid,  
Filamentous,  
Irregular raised )

Small Colonies

(circular,  
punctiforms ,flat,  
convex, entire,)

Large colonies,

regular, colored or  
not.



**Molds  
stain**

**mold Slide**

**Bacteria**

**Gram stain**

**Yeast  
simple**