***2- Viable count (Indirect microscopic count of bacteria).***

1. Pour Plate Technique

The Pour Plate Technique can be used on any type of liquefied sample for

the enumeration of bacteria . Conditions vary depending upon the type of

bacteria being enumerated . Only live bacteria are counted in this method.

Procedure :

1- Take 10 fold serial dilution of sample

2- Take 10 test tubes. 9 ml of any diluents is taken in each test tube e.g.

normal saline .

3- 1 ml of sample is poured first in first test tube by a pipette and it is

then mixed thoroughly . (dilution 1/10)

4- 1 ml is transferred from first test tube to second test tube by pipette.

It is mixed again . (dilution 1/100)

5- In the same pattern dilution is done up to the last test tube (in this

fashion as dilution is increasing, bacterial number is decreasing ).

6- 10 Petri dishes are prepared now with general purpose nutrient agar

in each, then held at 44-46°C in a water bath .

7- 1.0 mL of the sample or dilution is transferred to a sterile, empty

petri dish from each test tube is done in each respective plate . Agar

is melted by heating in boiling water, and then allowed to cool in a

water bath to 44-46°C

8- Approximately 15 mL of agar medium is poured into the petri dish

containing the sample . The sample and agar are mixed thoroughly

by rotating the plate several times .

9- When the media has solidified , the plates are inverted and incubated

10- Incubate these plates at 37°C for 24 hours Dense colonies are

formed in first two plates and then gradual decrease in intensity is

seen .

11- Select only one plate having 30-300 colonies and count the number

of colonies in it e.g. plate number 4 is giving 240 colonies . (As a

colony is formed by a single living cell, so a colony represents a

living cell ).

Formula :-

The number of cells /ml = average number of colonies apparent **×** dilution

inverse

Law of the dilution is uses :

Additive / Additive + present x the previous dilution

***B- Spread Plate Technique***

Standard Methods Agar (SMA) is used routinely for the spread plate

technique to enumerate aerobic bacteria .

1- Plates are allowed to warm to room temperature and dry before

inoculating .

2- Serial dilutions are prepared (using 0.1 ml) so that following

incubation on nutrient agar or any media , and spreader is uses to

bacteria spreading on the plate , one of the dilutions will yield

growth of 30-300 colonies (the ideal range for counting ) on the

agar plate .

3- The plate is inoculated from the dilution (which has been

thoroughly mixed), or directly from the sample using a 0.1 ml

inoculum , if low counts are expected.

4- The inoculum is transferred onto the agar surface near the center if

the plate is spread , or at a designated mark on the plate if it is

being spread by an automatic spreading device .

The inoculum is spread over the surface (by spreader) and allowed to be

absorbed by the medium . Plates are inverted and incubated as follows :-

Marked dishes and placed inverted and incubated at 37°C and after

incubation law is used :

The number of cells /ml = number of colonies apparent \* inverse dilution

\* 10

