Mi5 : Fecal contamination indicators : thermoduric and total coliform and streptococci in surface water

MPN technique
Necessary Document : Mc Grady Table

1. Principle

FCI are researched in surface water as contaminants issued from waste water or other contaminated discharge ; these waters are turbid and the numeration can't be performed by means of filtration.
The following statistic method is applied :
Sets of sterile nutritive containing tubes are inoculated by different serial dilutions of the tested water ; once a high enough dilution is inoculated, only one bacterium is introduced in the tube, and after incubation, the growth is detected as the medium aspect turns (becomes turbid…)

The further dilutions don't incorporate any bacterium in the tubes and they remain sterile.

At the end of the incubation, we note the number of positive tubes, per dilution.

A statistic table, called Mac Grady Table, and by means of the precedent result, indicates us a number, the MPN ; a formula allows us to calculate the concentration of bacteria in our sample.

Example of result : inoculation of set of **three** tubes with serial dilution of the sample

Dilution	10^1	10^2	10^3	10^4	10^5	10^6
Result : number of positive tubes	3	**3**	**2**	**1**	0	0

Highest dilution of the three

Characteristic Number = 321 (see the Mac Grady Table rules)

For 321, the Table indicate
MPN = 15
Then the concentration is $15x10^{\text{higest dilution}}$ / 100 mL = $15x10^4$ / 100mL. (150,000)

The used medium for determination of coliform by MPN technique is called BLBVB ; this selective broth contains chemicals that inhibit cocci Gram positive growth (like streptococci…)
In the same way, selective Litsky broth contains chemicals that inhibit rod shaped Gram negative (like coliforms…) growth.
There should be two steps : presumption and confirmation ; we only use confirmative broth.

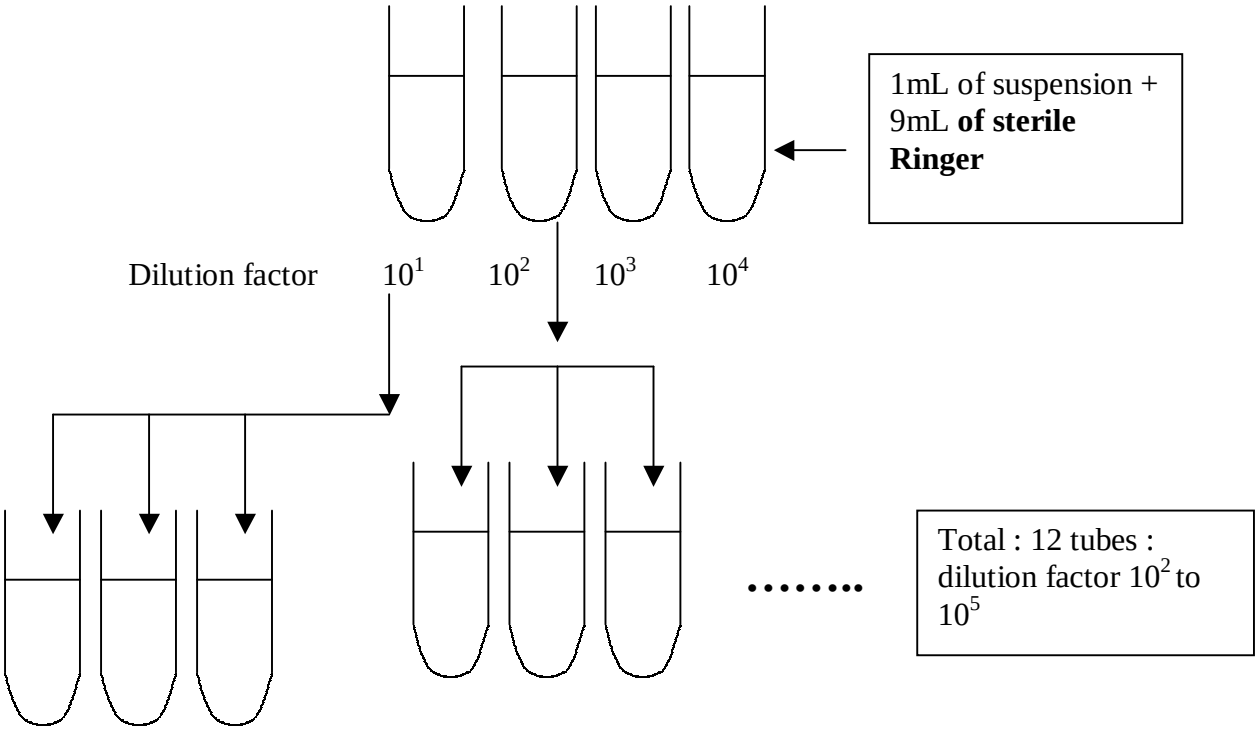
2. Operation

Requirements for one experiment:

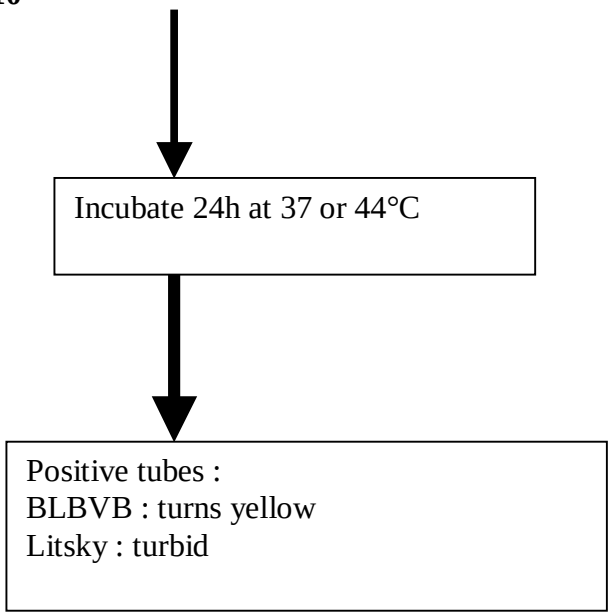
One flask of 100mL of raw water or of a suspension containing
10^5 E.coli / 100 mL *(10^3 / mL) labelled « coli »*
One flask containing 100 mL of
10^5 Enterococcus faecalis / 100 mL *(10^3 / mL) labelled « entero »*
4 tubes of 9mL sterile ringer
12 tubes of 9 mL BLBVB broth
12 tubes 9 mL Litsky broth
12 sterile pipettes of 1 mL or 2 mL

Carry out a first set of water sample dilutions by means of the 10 tubes containing 9mL of sterile Ringer :
Page 1 : pipetting helpers and how to use a pipette
Page 2 : the use of a pipette pump
Page 3 : General steps in a dilution procedure *(the method, i.e. Serial dilution and spread technique, will not be used for this lesson)*

Sketch :



First set of 3 tubes containing selective broth :
BLBVB for coliform
Litsky for streptococci
Dilution factor : 10^2



3. Report

Indicate your result in a table (+++ , ++- , +-- , ---), the characteristic number (here : 321), the MPN and the bacteria concentration of the sample.