Mi6 : disinfection

1. Requirement for one experiment (one group) : testing one disinfection time

- one 200 mL erlen flask containing 1500 E.coli / mL in Ringer
- 13 tubes containing 10mL of sterile Ringer + 3 for initial suspension numeration
- one 50 mL flask containing Bleach exactly 150 mg / L
- one 50 mL flask containing $Na_2S_2O_3$ 0.0028 mol / L
- 26 tubes containing 20 mL of melting agar (80C) + 3 for initial suspension numeration
- 26 sterile empty Petri dishes + 3 for initial suspension numeration
- 16 sterile 1mL - pipette
- one 0-20µL micropipette, one 0-100 and 0-1000 and sterile tips
- one stop watch

2. Generalities

Watson Chick law governs the action of disinfectants

$C^n . t = - 1/A \ln (Nt/No)$

C is the disinfectant concentration (mg/L)

t is the action time (min)

No is the initial bacteria concentration

Nt is the final bacteria concentration

Nt/No is the kill rate

A is a constant depending on the micro-organism

N is the dilution constant :

If n >1, the disinfectant concentration is more important than the action time

If n < 1, the action time is more important

If n=1, the ln of the kill rate is proportional to the product CT : the kill rate logarithm will Double either one double the time or double the concentration

The product CT_{99} , i.e. the CT value to reach in order to obtain a kill rate equal to 0.99, is equal to 0.005mg / L / min for chlorine and E.coli.

3. Operation

The aim of the lesson is to determinate :

- the two constants n and A
- the CT value in order to obtain a 0.99 kill rate

We-ll apply different chlorine concentration to a bacteria suspension (0 to 1 mg / L) and for a constant time (30s, 1 min, 1min and 30s, 2 min)

Chlorine action is stopped by neutralization with thiosulphate

The chlorinated suspension is inoculated in plate agar (2) in order to count bacteria after incubation : **pour plate technique above**

Bacteria are counted before and after each disinfection rate.

General organization : groups of 3 students ; each group tests one action time

Tube n°	0	1	2	3	4	5	6	7	8
Vculture mL	10								
TT_{Cl2} $mgCl_2.L^{-1}$	0	0.05	0.1	0.15	0.20	0.25	0.3	0.4	0.5
V bleach $_{150\ mg\ /\ L}$ (µL)	0	3.25	6.5						32.5
Gently mix and start stop watch : after requested time, add 100 µl of sterile 0.0028 thiosulphate									
Dilution to inoculate: **See above**: pour plate technique	1, 10 and 100	1	1	1	1	1	1	1	1

Tube n°	9	10	11	12	13
Vculture mL	10				
TT_{Cl2} $mgCl_2.L^{-1}$	0.6	0.7	0.8	0.9	1
V bleach $_{150\ mg\ /\ L}$ (µL)					65
Gently mix and start stop watch : : after requested time, add 100 µl of sterile 0.0028 thiosulphate					

Dilution to inoculate: **See above**: pour plate technique		1	1	1	1	1

Pour-plate technique :

Place nutrient agar deeps into the boiling water bath for melting.
Remove this tube and cool to 45°C .
Introduce, with a sterile transfer pipette, 1 mL of the sample of tested water in the bottom of a sterile Petri dish

Pour the liquid agar medium (40 to 50 °C) in this Petri dish and rotate gently in order to obtain a regular repartition of the colonies ; harden the medium on a horizontal and cold surface.
Label the side of the Petri dish (name, date, temperature of incubation).
Turn over the Petri dish and incubate your pour-plate at 22°C during 72h or at 36°C during 24h.

Numeration of the colonies:
Consider the Petri dish which present less than 300 colonies.

Each colony has been formed from the development of a unique cell.

If N is the number of colonies counted in a Petri dish containing the dilution 10^n :

C (microorganisms / mL) = $N / 10^n$

Ex :
a) 102 colonies counted in the Petri dish seeded with non diluted sample of tested water :
$C = 102 / 10^0$ = 102 microorganisms / mL
b) 68 colonies counted in 10^{-4} dilution plate (1 : 10,000) :

$C = 68 / 10^{-4}$ = 680,000 microorganisms / mL

☞ one colony can be formed from the development of two cells or more: sometimes, the viable number is expressed in terms of colony forming units (CFUs) : 102 or 680,000 CFUs / mL.

☞ if you seed two agar plates per dilution, you can add the number of colonies and divide this number by the sum of the poured volumes:

Dilution / **poured volume equivalent (mL)**	**Number of colonies in plate 1**	**Number of colonies in plate 2**	**conclusion**
10^0 / 1	*Too numerous to count*	*Too numerous to count*	*Non valid (>300)*
10^{-1} / 0.1	*332*	*303*	*Non valid*
10^{-2} / 0.01	**45**	**51**	**96**
10^{-3} / 0.001	**6**	**4**	**10**
10^{-4} / 0.0001	**1**	**1**	**2**

C = [96+10+2] / (0.01 + 0.001 + 0.0001) = 9729 CFUs / mL

4. Report

Indicate in a table all your results, i.e. the bacterial counts
Save the other groups results
Indicate the initial bacteria concentration
Plot ln (N_t /N_0) = f(chlorine rate) for each tested time
Calculate A and n
Calculate the CT value such as the kill rate is equal to 0.99