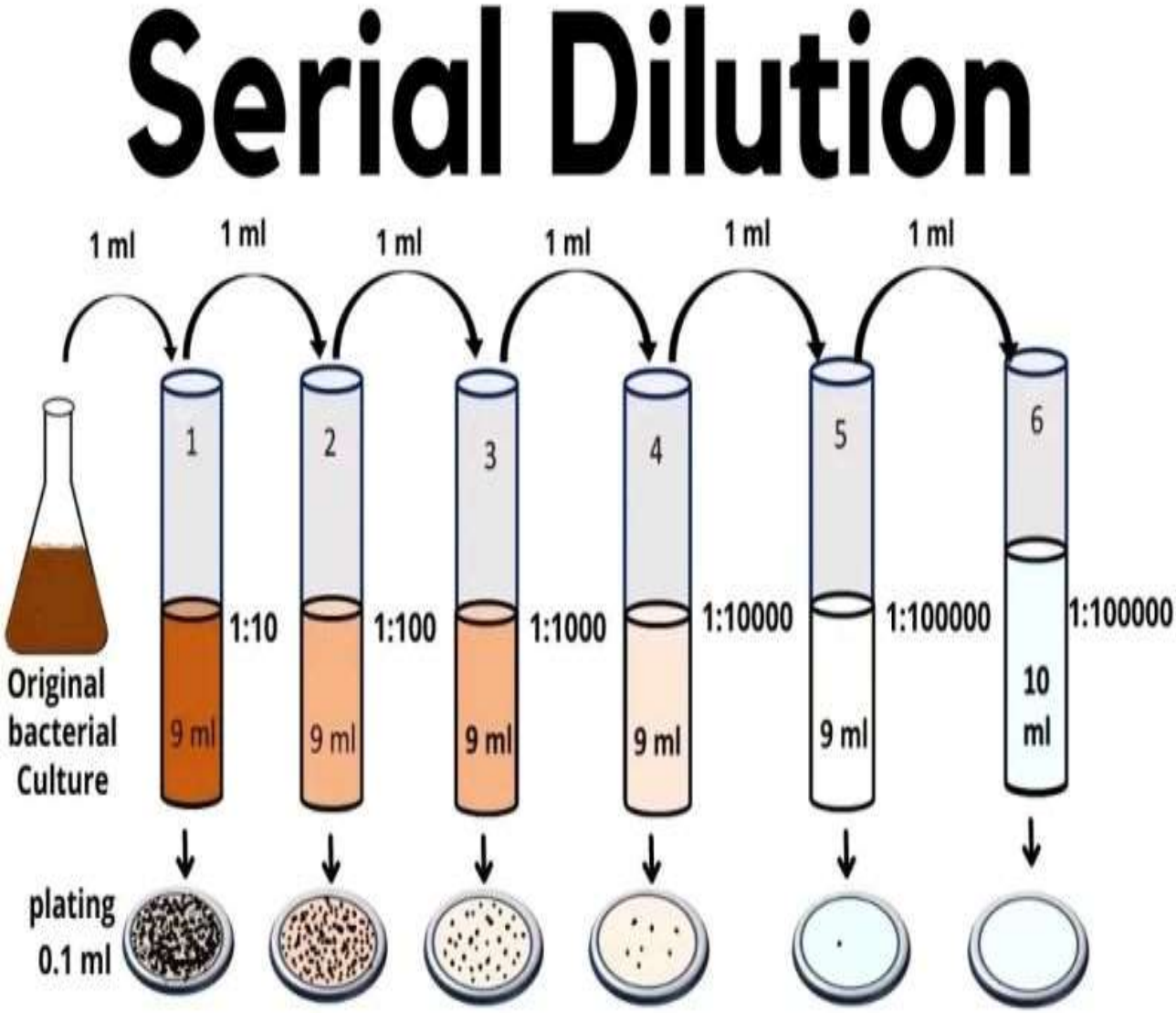
Lab-2 Practical Bacterial physiology

A ten-fold dilution

A ten-fold dilution, also known as a **1:10 dilution**, reduces the concentration of a solution or substance by a factor of ten. This means the new concentration is one-tenth of the original concentration. It's a common technique in scientific experiments, especially when dealing with **very high concentrations.**

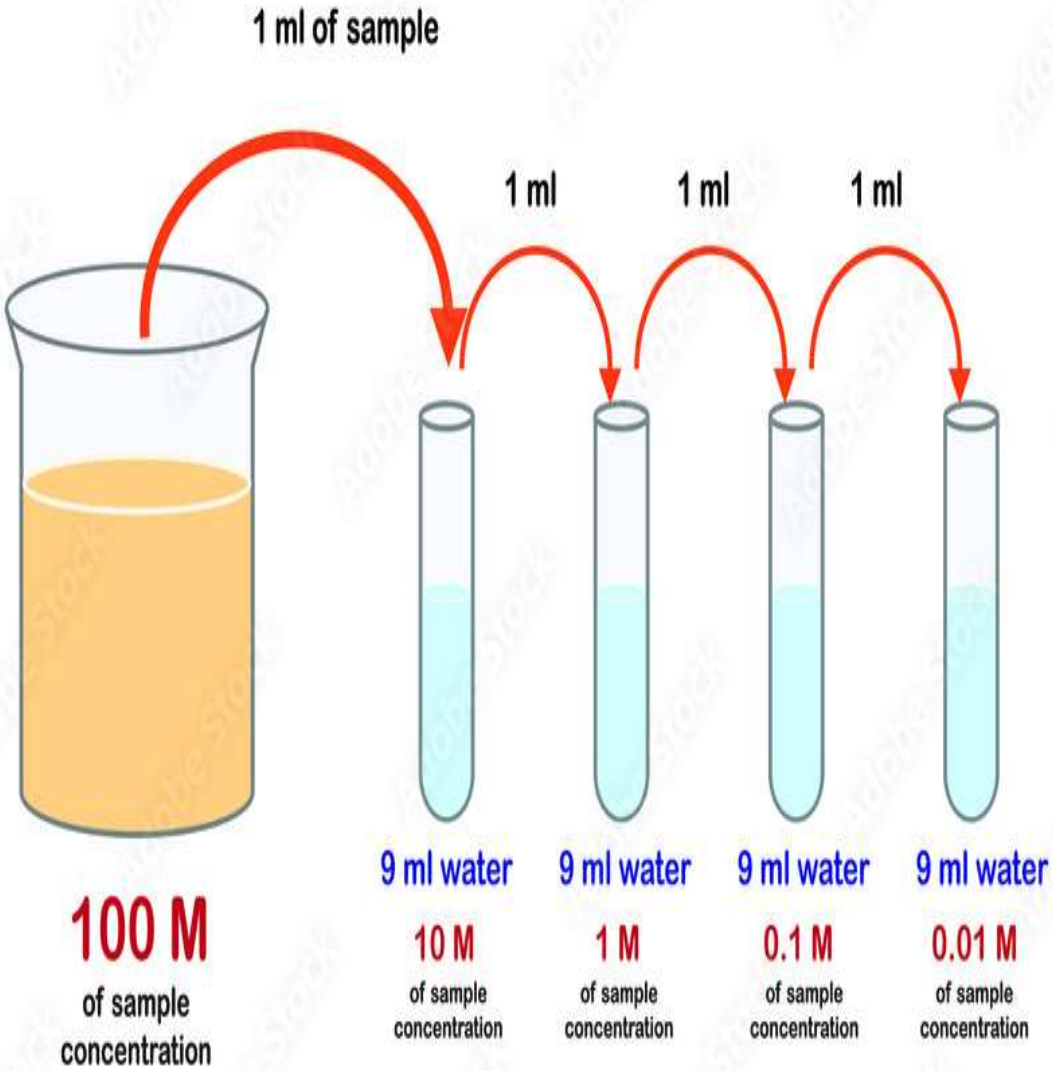
- **Ratio:** A ten-fold dilution involves a 1:10 ratio, meaning 1 part of the original solution is mixed with 9 parts of diluent (**water** or **buffer**) to create a total volume that is ten times larger.
- **Concentration Reduction:** Each ten-fold dilution reduces the concentration by a factor of ten.
- **Example:** If you start with a **1 M solution** and perform a ten-fold dilution, the resulting solution will have a concentration **of 0.1 M** (1/10 of the original).

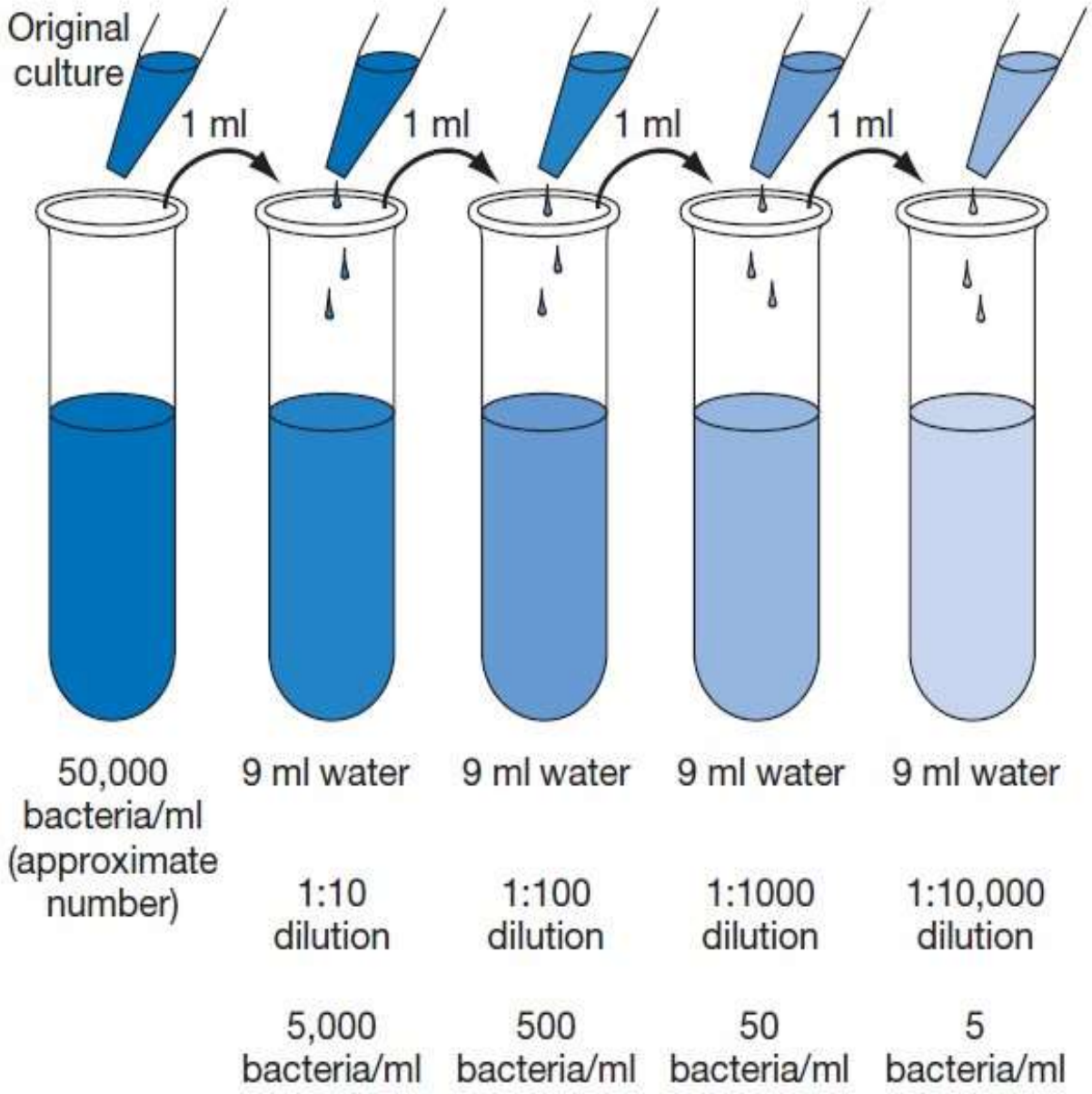
Serial Dilutions: When multiple ten-fold dilutions are performed in a series, it's called **a ten-fold serial dilution**. This is often used to achieve a much lower concentration from a high starting concentration in a few steps.



Science Experiment

SERIAL DILUTION




Original culture
1 ml
1 ml
1 ml
1 ml
50,000 bacteria/ml (approximate number)
9 ml water
1:10 dilution
5,000 bacteria/ml
9 ml water
1:100 dilution
500 bacteria/ml
9 ml water
1:1000 dilution
50 bacteria/ml
9 ml water
1:10,000 dilution
5 bacteria/ml

Understanding Basics of Dilution

Let's say you have a glass of sugar solution at 6.0 M concentration and another glass with water. If you took 1 mL of sugar solution and dispensed it in a new tube, then added 9 mL of water and mixed. You just made a dilution.

To calculate the dilution factor (df), you need to divide the solution's total volume by the sample volume (i.e., sugar solution in this case).

Total volume = (Volume of sample + Volume of diluent), i.e., 1 mL sugar solution + 9 mL water = 10 mL

$$\text{Dilution factor} = \frac{\text{Total volume}}{\text{Sample volume}} = \frac{10\ \text{mL}}{1\ \text{mL}} = 10$$

Dilution Factor = Total volume/Sample volume = 10/1= 10

This dilution can be expressed by various terms like

- **The dilution factor is 10 (dilution factor is the reciprocal of the dilution)**
- **It was a 10-fold dilution**
- **It was diluted by 1/10.**

Calculating the concentration

To calculate the concentration of this diluted sample, multiply by the inverse of the dilution factor.

Final concentration = (Initial concentration X 1/ dilution factor), i.e., 0.6 M

$$\text{Final concentration} = \text{Initial concentration} \times \frac{1}{\text{Dilution factor}}$$

$$= 6M \times \frac{1}{10}$$

$$= 0.6M$$

Sometimes, you may wish to calculate backward. Let's say you have a sample that has been diluted 1/10 with a concentration of 0.6 M and wants to calculate its undiluted concentration.

Undiluted concentration = (Diluted concentration X dilution factor)

= 0.6 M *10 = 6M.

Multiple or Serial Dilution

Let's say you again diluted the sugar solution by taking 1 ml of diluted sugar solution in another 9 mL of water. Your final dilution ends up being 1/10 X 1/10 = 1/100 dilution. In other words, your sample has been diluted 100-fold (d.f. =100).

If you want to measure the concentration of the final solution after two successive 10-fold dilutions, follow the same formula: (6 M X1/10 X 1/10) = 6/100 = 0.06 M

So, If you need 100-fold (10^{-2}) dilution, you can either add 0.1 mL sample with 9.9 mL of diluent or make two successive 10-fold dilutions.

Preparation of diluent

1. Prepare six test tubes that can store 20 mL or more in a rack and label them T1-T6.
2. Each tube is consistent with the dilution factor it corresponds to (i.e., T3= 1×10^3 OR 0.001th of stock concentration).
3. Pipet 9 mL of sterile water, saline, or broth into each of the 6 test tubes.
4. Sterilize the diluent banks by autoclave. Use aluminum foil to cover each of the six test tubes and then transfer them to an autoclave-compatible test tube rack. Sterilize for a minimum of 15 minutes at 121°C, 15 PSI.
5. Remove blanks using heat-resistant gloves and allow to cool. When tubes reach room temperature, cover and store at 4°C until needed.

Serial Dilution Method

1. Obtain the sample flask from the incubator and shake it vigorously.
2. Pipet 1 mL of “solution” into the test tube labeled T1. Vortex T1.
3. Remove 1 mL from test tube T1 and add it to test tube T2. Vortex T2.
4. Remove 1 mL from test tube T2 and add it to test tube T3. Vortex T3.
5. Remove 1 mL from test tube T3 and add it to test tube T4. Vortex T4.
6. Remove 1 mL from test tube T4 and add it to test tube T5. Vortex T5.
7. Remove 1 mL from test tube T5 and add it to test tube T6. Vortex T6.

Uses of Serial Dilution Method

Serial dilution is one of the most important skills a biology graduate must develop. It has wide applications in various disciplines of biology. It is used for isolating and quantitating the number of microorganisms present in various samples such as water, food, soil .

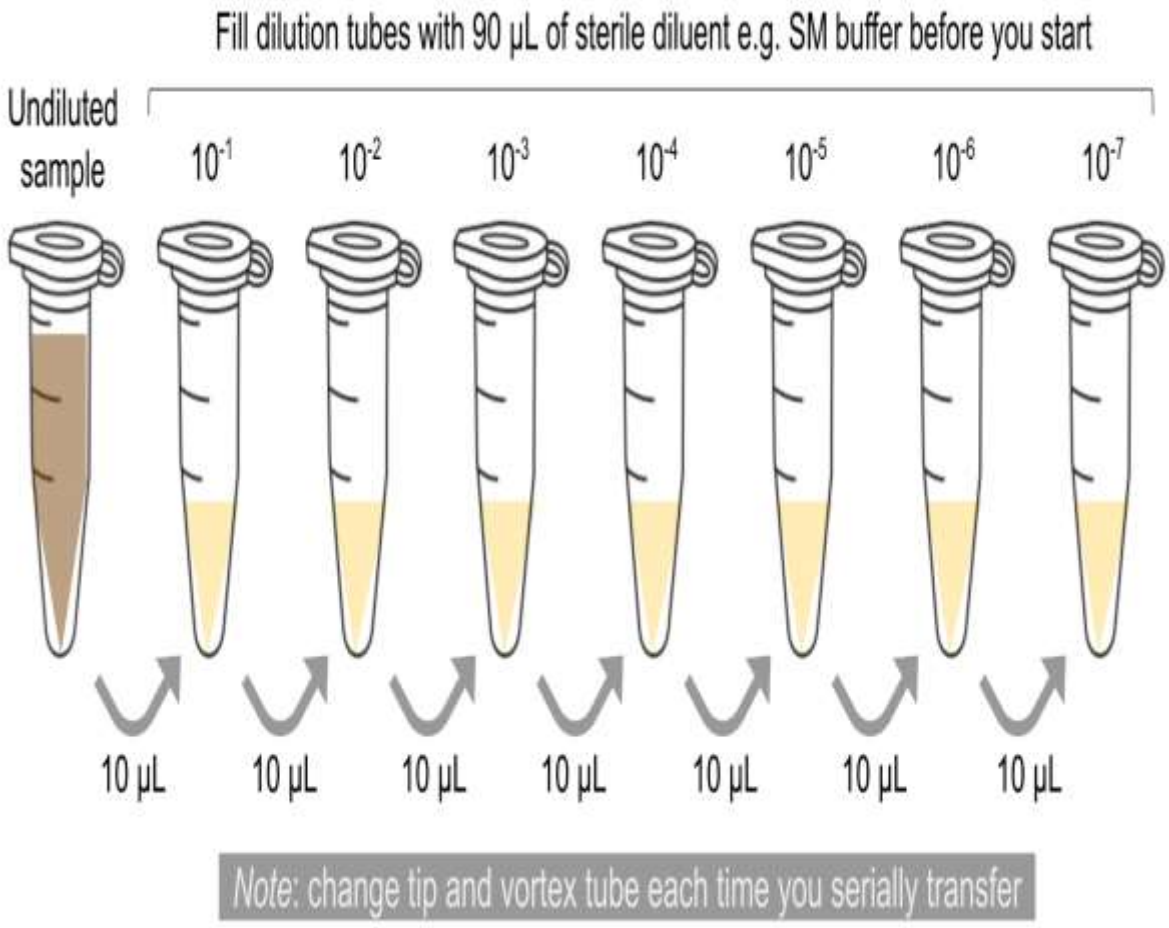
Serial dilution skill is required for bioburden testing

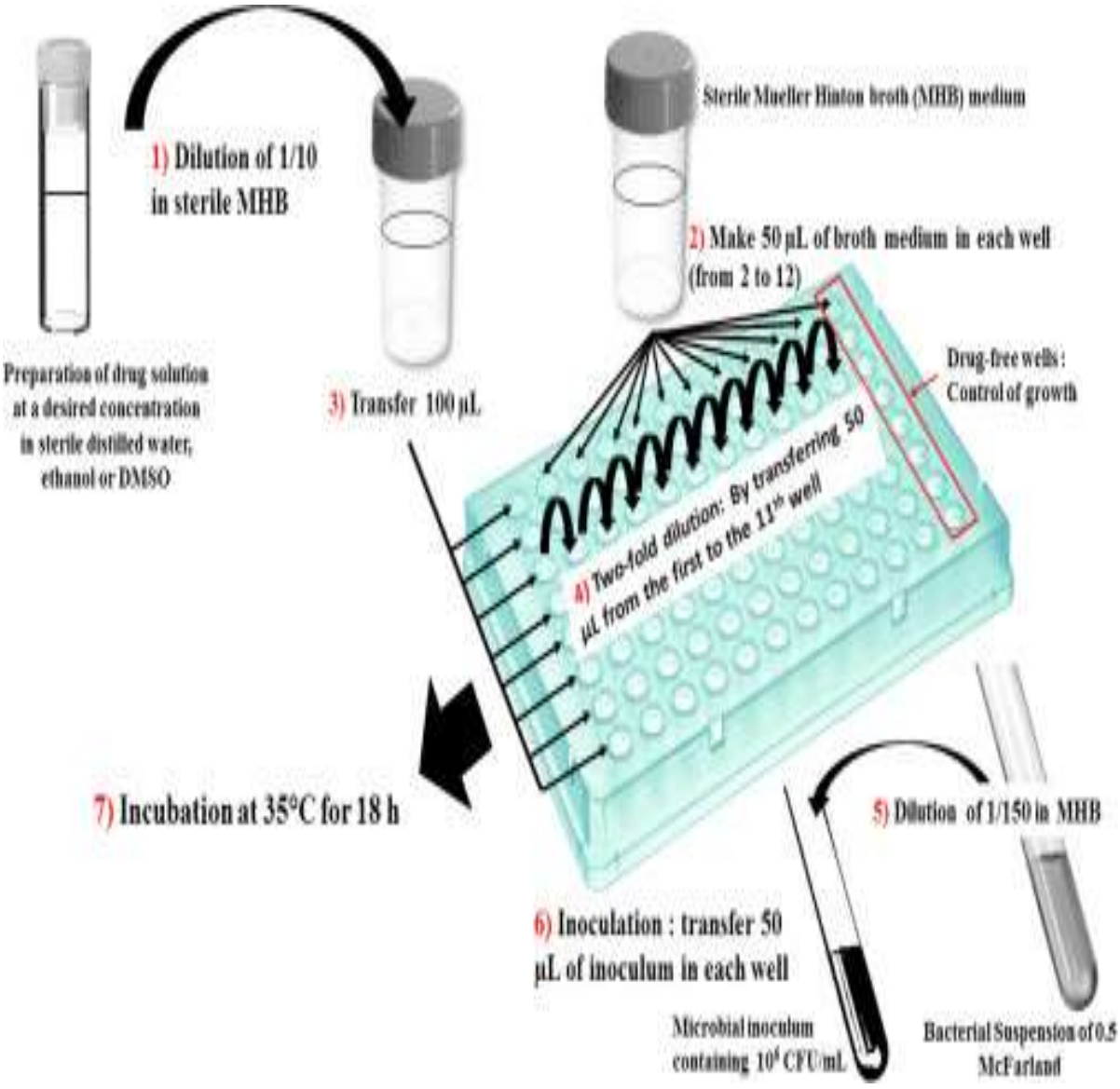
1-minimum inhibitory concentration (MIC).

2-most probable number method (MPN).

3-determination of antibody titer.

4-determination of minimum lethal dose (MBC).


Fill dilution tubes with 90 µL of sterile diluent e.g. SM buffer before you start
Undiluted sample
10-1
10-2
10-3
10-4
10-5
10-6
10-7
10 µL
10 µL
10 µL
10 µL
10 µL
10 µL
10 µL
Note: change tip and vortex tube each time you serially transfer


1) Dilution of 1/10
in sterile MHB
Sterile Mueller Hinton broth (MHB) medium
2) Make 50 µL of broth medium in each well
(from 2 to 12)
Preparation of drug solution
at a desired concentration
in sterile distilled water,
ethanol or DMSO
3) Transfer 100 µL
Drug-free wells :
Control of growth
4) Two-fold dilution: By transferring 50
µL from the first to the 11th well
7) Incubation at 35°C for 18 h
5) Dilution of 1/150 in MHB
6) Inoculation : transfer 50
µL of inoculum in each well
Microbial inoculum
containing 10⁵ CFU/mL
Bacterial Suspension of 0.5
McFarland