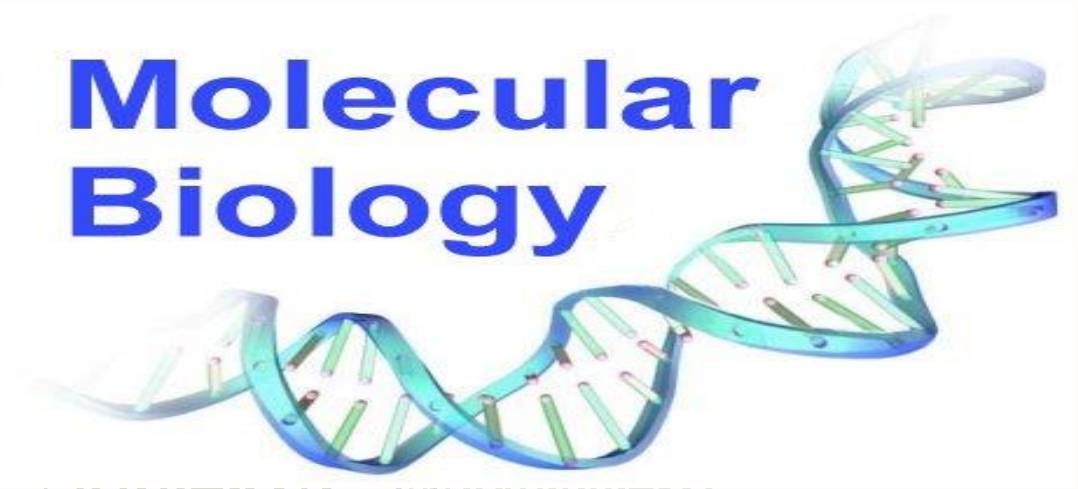

جامعة الملك سعود
King Saud University
1957

Serial Dilution

Vortex mixer

Spectrophotometer

251 MIC.

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Roua AL Kufeidy

How to do serial of dilution?

A dilution definition is:

a process that reduces the concentration of a substance in a solution.

A serial dilution definition is:

the repeated dilution of a solution to amplify the dilution factor quickly

In which experiment do we often need dilutions?

- It’s commonly performed in experiments requiring highly dilute solutions with great accuracy.

such as involving experiments to determine density of bacteria.

How to do serial dilution?

1- Determine the proper dilution liquid

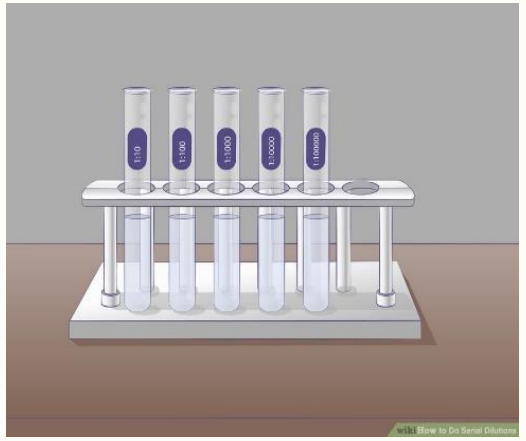
distilled water or culture media

2- Prepare several test tubes with 9 mL of dilution liquid

- You will be adding your undiluted sample to the first tube and then serially diluting into the following tubes.
- label all of your tubes
- Each tube will be a 10-fold dilution starting from the undiluted tube
- The first tube will be a 1:10 dilution…..etc.

3- Prepare a test tube with at least 2 mL of your undiluted solution

- to perform this serial dilution is 1 mL of undiluted solution
- Thoroughly mix your solution before starting any dilutions



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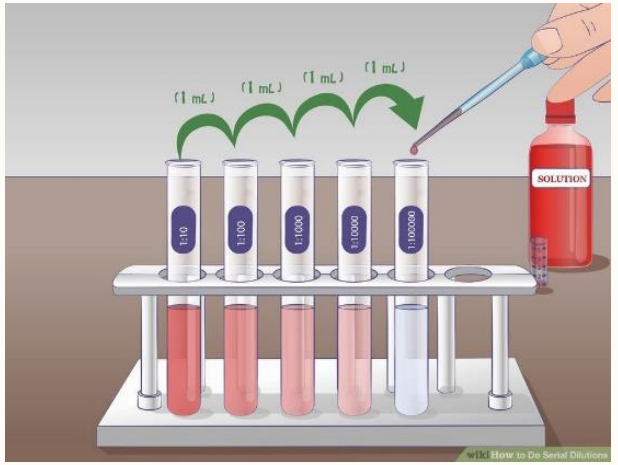
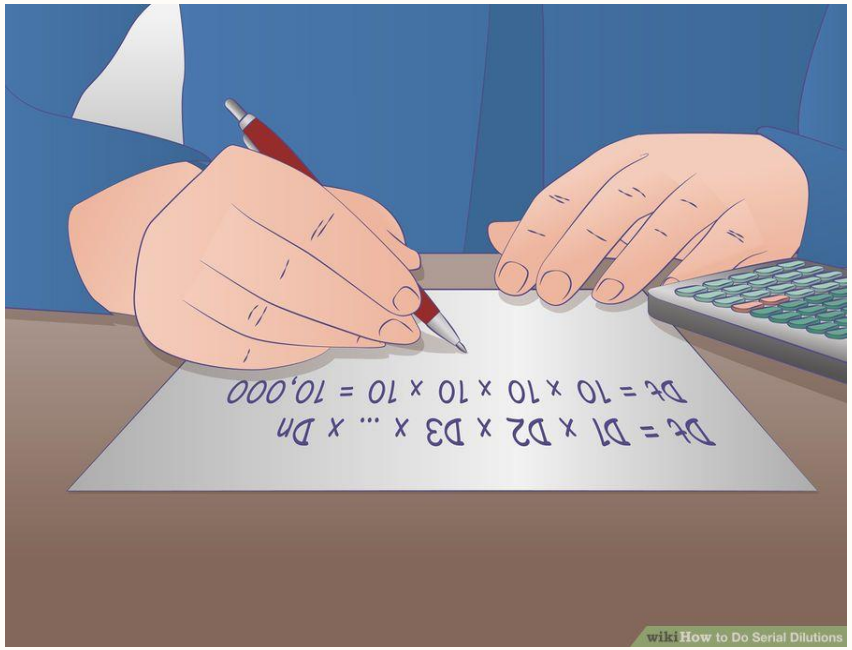
How to do serial dilution?

4- Perform the first dilution. Draw 1 mL of undiluted solution from test tube with a **micropipette** and transfer it to the test tube labeled *1:10* containing 9 mL of the dilution liquid and mix thoroughly
The solution, therefore, has been diluted by a factor of 10

5- Perform the second dilution. For the second serial dilution, you will take 1 mL of solution from tube *1:10* and add it to the 9 mL of dilution liquid in the tube *1:100*. Thoroughly mix tube *1:10* before adding to the next tube.



How to do serial dilution?



6- Calculating Final Dilution Factor and Concentration

•Concentration definition:

the amount of solute dissolved in a volume of solution.

(1) volume of solution increases from V_{intial} to V_{final}

(2) concentration of solution decreases from $c_{initial}$ to c_{final}

•Dilution factor:

refers to the ratio of the volume of the initial (concentrated) solution to the volume of the final (dilute) solution, that is the ratio of V_1 to V_2 or, $V_1 : V_2$

•A dilution factor, DF, can be calculated:

$DF = V_2 \div V_1$

How to do serial dilution?

•Example:

•prepare a 1:50 dilution of the solution.
take a known volume of the stock solution ($V_{initial}$) and add enough solvent to it so that the solution has a new volume, V_{final}
of 50 x $V_{initial}$.
•V_{final} = 50 x $V_{initial}$
•The "1:50" tells you the dilution factor, the ratio of volumes, to use to prepare the new solution.
•V1:V2
•1:50
•In this case it tells us that V_1 = 1 and V_2 = 50
so the dilution factor, DF, = $V_2 \div V_1$ = 50 ÷ 1 = 50
That is, the new, diluted solution will have a volume 50 times greater than the volume of the original, undiluted, solution:

V_{final} = DF x $V_{initial}$

How to do serial dilution?

The table below gives you a number of different options for preparing a 1:50 dilution:

V_{intial} (initial volume)	1 mL	1 L	0.1 mL	2 mL	25 µL
V_{final} (final volume)	50 mL	50 L	5 mL	100 mL	1250 µL
ratio of volumes used $V_{initial} : V_{final}$ $V_1 : V_2$	1 : 50 1 : 50	1 : 50 1 : 50	0.1 : 5 1 : 50	2 : 100 1 : 50	25 : 1250 1 : 50
dilution factor $DF = V_2 \div V_1$	50 ÷ 1 = 50	50 ÷ 1 = 50	50 ÷ 1 = 50	50 ÷ 1 = 50	50 ÷ 1 = 50

How to do serial dilution?

7- Determine the concentration of the solution following dilution

The equation is $C_{final} = C_{initial}/D$ where C_{final} is the ending concentration of the diluted solution, $C_{initial}$ is the starting concentration of the original solution and D is the dilution ratio previously determined

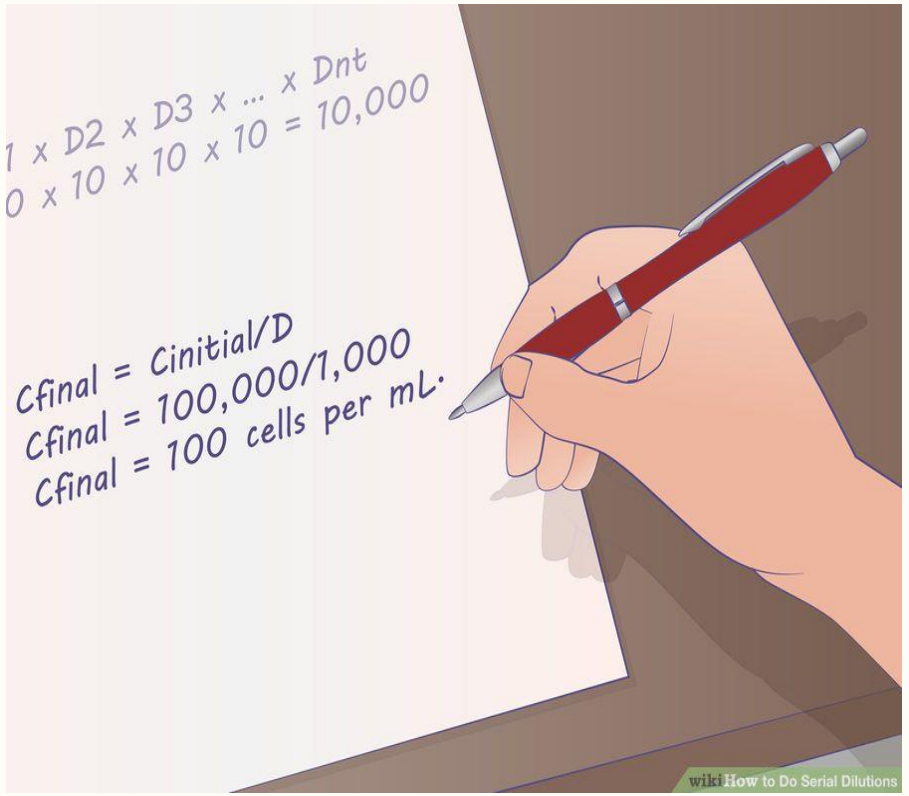
Example: If you started with a solution of cells with a concentration of 1,000,000 cells per mL and your dilution ratio is 1,000, what is the final concentration of your diluted sample?

Using the equation:

$C_{final} = C_{initial}/D$

C_{final} = 1,000,000/1,000

C_{final} = 1,000 cells per mL.



Vortex Mixer

Vortex: - is a simple device used commonly in laboratories to mix small vials of liquid. As the motor runs the rubber piece oscillates rapidly in a circular motion.

- When a test tube or other appropriate container is pressed into the rubber cup (or touched to its edge) the motion is transmitted to the liquid inside and a vortex is created.

- **Vortex mixers** are quite commonplace in bioscience laboratories.

- In cell culture and microbiology laboratories they may be used to suspend cells. In a biochemical or analytical laboratory they may be used to mix the reagents of an assay or to mix an experimental sample and a dilutant.

Vortex mixer

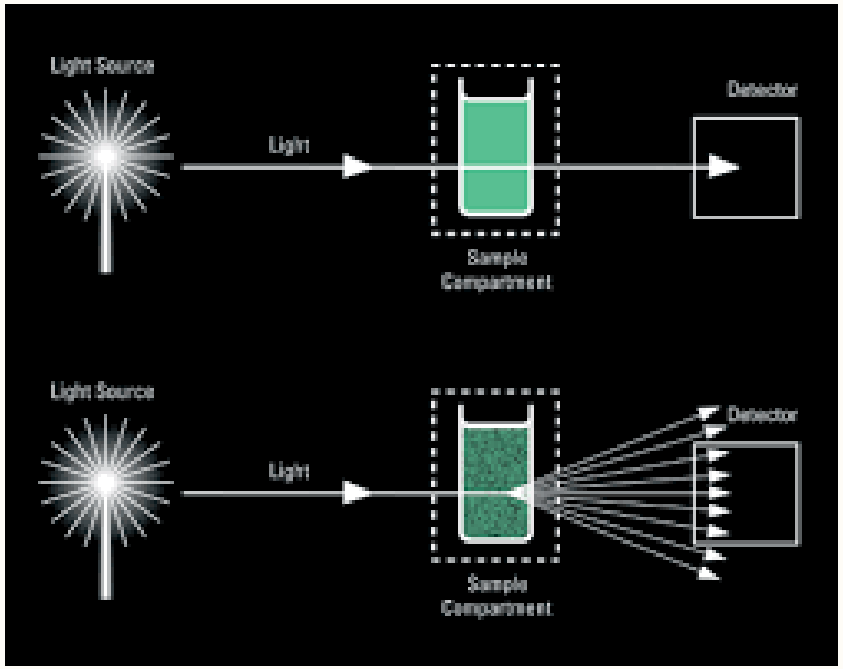
Heated Microplate Vortexer

Spectrophotometer

A spectrophotometer is

- commonly used for the measurement of transmittance or reflectance of solutions, transparent or opaque solids, such as polished glass, or gases.
- to measure the diffusivity on any of the listed light ranges that usually cover around 200 nm - 2500 nm using different controls and calibrations.

Calibration in measurement technology and metrology is the comparison of measurement values delivered by a device under test with those of a calibration standard of known accuracy.



Spectrophotometer

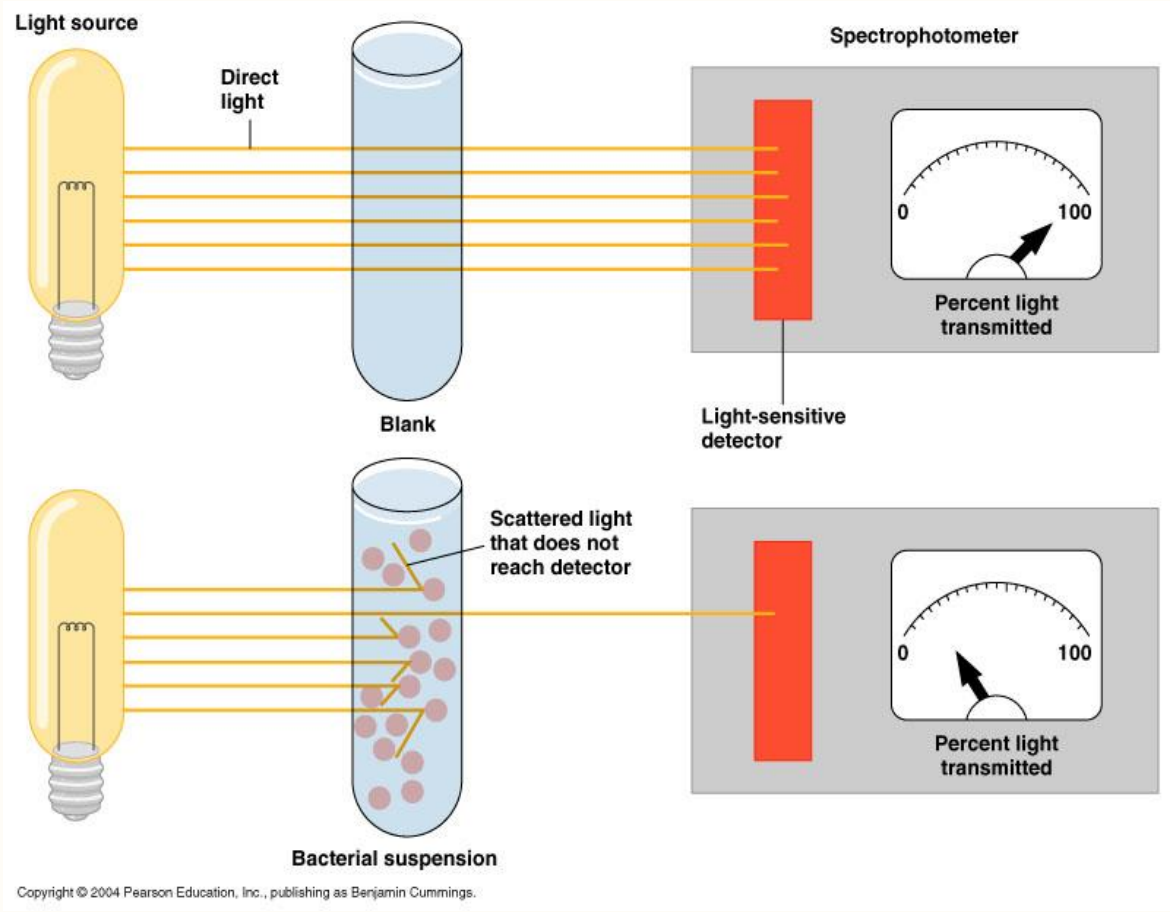
Estimating Bacterial Numbers by Indirect Methods

A spectrophotometer is used to determine turbidity ("cloudiness") by measuring the amount of light that passed through a suspension of cells.

More cells = more turbidity; more turbidity = less light passing through the suspension

%T is percent transmission - fewer cells present (less turbidity) will allow more light to pass through, the %T is higher when the cell number is lower.

Absorbance is the opposite of %T. More light is absorbed when more cells are present - some people like this measure better because absorbance goes up as turbidity (or cell number) goes up.



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Roua AL Kufeidy