

Antibiotic Technology Laboratory

Lab 1: Serial dilution

Why is it called an antibiotic?

What's in a Name? The term antibiotics literally means “against life”; in this case, against microbes. There are many types of antibiotics—antibacterials, antivirals, antifungals, and antiparasitics. Some drugs are effective against many organisms; these are called broad-spectrum antibiotics.

Serial dilution is a fundamental laboratory technique used to systematically reduce microbial concentration, allowing accurate and reliable colony enumeration. This stepwise dilution method is essential in microbiology, clinical diagnostics, food testing, and pharmaceutical quality control.

Sample: refers to the original liquid that will be tested

Diluent: the solution that is mixed with the sample (often-purified water).

Concentration: refers to how much of the sample is in a given test. For example, “40 to 1” (1/40) **dilution** means that for every 40 parts of water, there is 1 part of sample.

Purpose of Serial Dilution

- 1- To obtain countable bacterial colonies
- 2- To estimate microbial concentration (CFU/ml)
- 3- To study microbial growth and viability.

Preparation of Dilution Tubes

Each test tube contains a fixed volume of sterile diluent (commonly 9 ml of saline or broth). These tubes are labeled according to the dilution factor (1:10, 1:100, 1:1000, etc.).

Stepwise Dilution Process

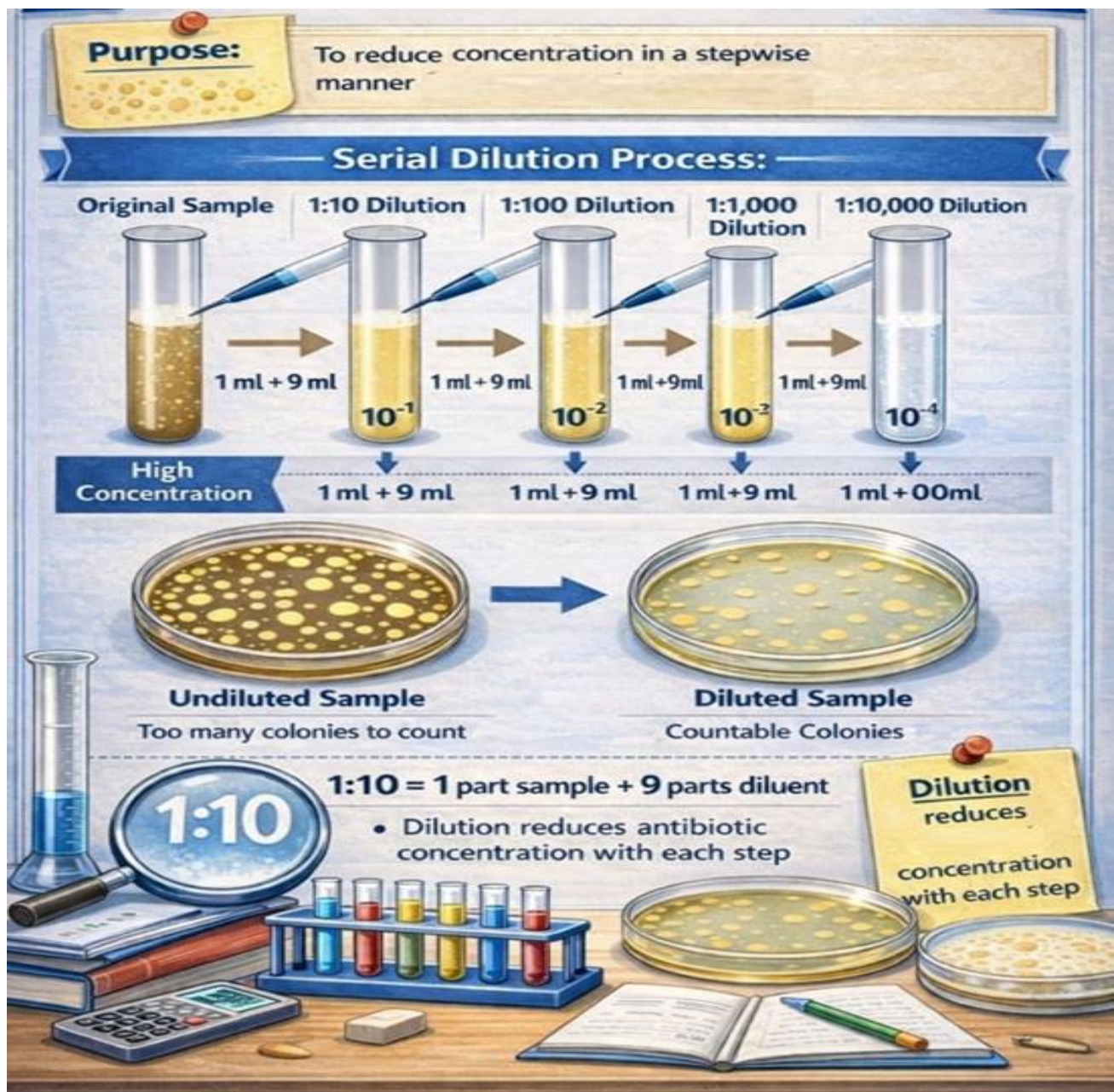
1 ml of the original culture is transferred into the first tube containing 9 ml diluent → 1:10 dilution

1 ml from the first tube is transferred to the second tube → 1:100 dilution

This process continues sequentially, producing 1:1000, 1:10,000, 1:100,000 dilutions

Each step reduces the concentration by a factor of ten.

Plating of Diluted Samples. From selected dilution tubes, a small volume (usually 0.1 ml) is spread onto agar plates. This allows individual bacterial cells to grow into visible colonies.



Type of dilutions:

- 1) **Decimal Dilution Method or One – Ten D.M.:** We transfer 1 ml of the original solution into the tube with 9ml of water, to become one part to 9 part.

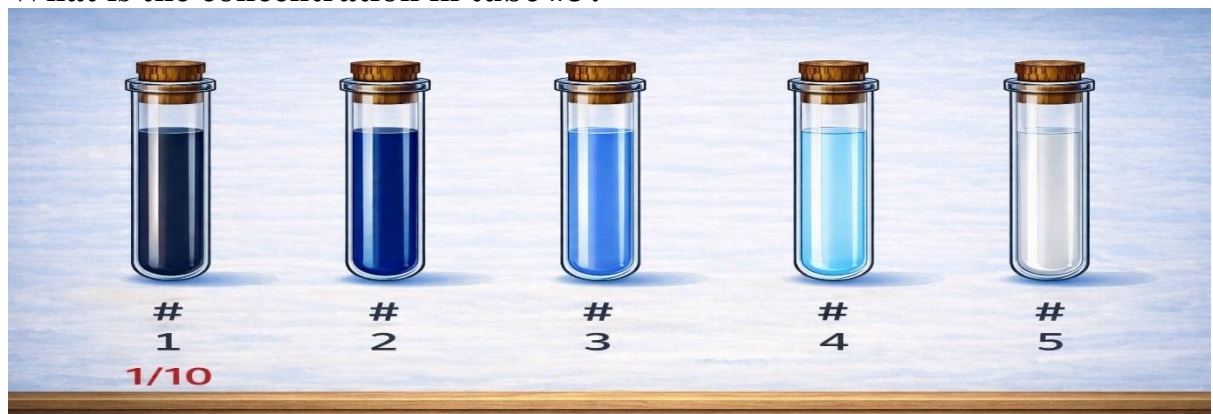


Always Remember:

1. Concentration $\neq$ Volume
2. Follow the formula

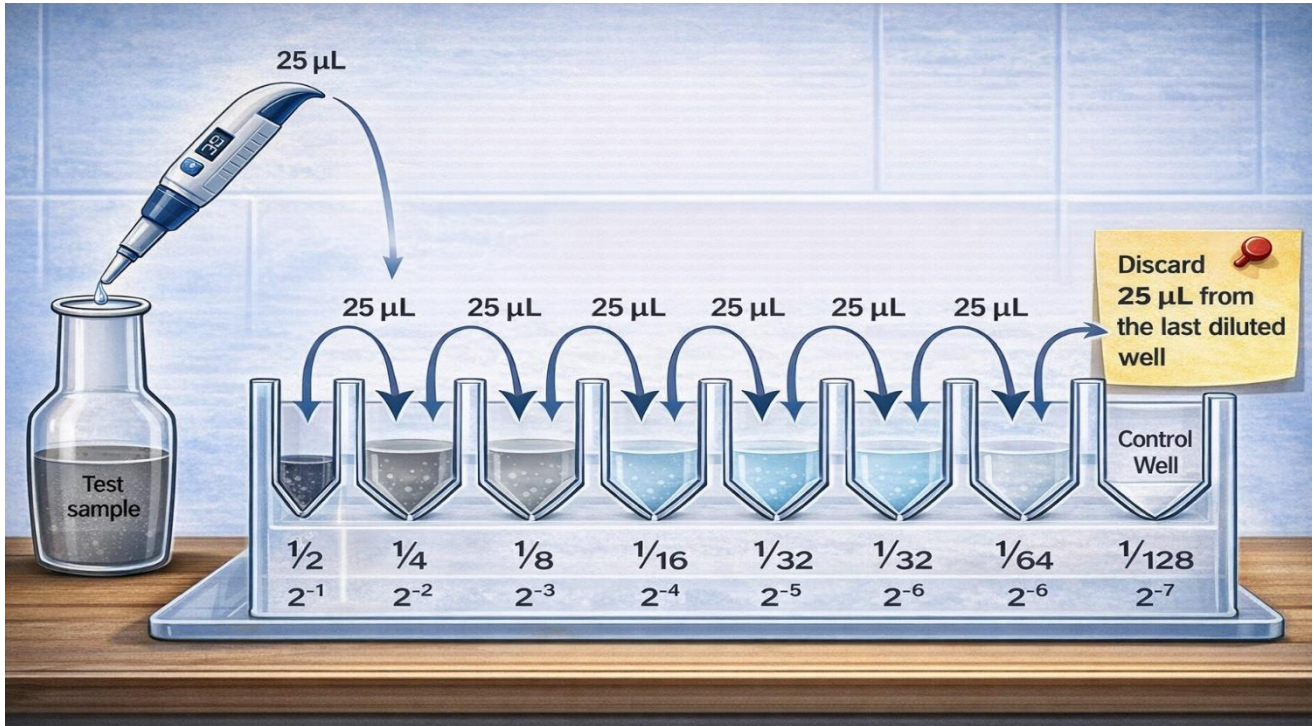
$$\text{Previous concentration} \times \frac{\text{Units transferred}}{\text{Total units}} = \text{New Concentration}$$

What is the concentration in tube #3?



2) **Two – fold Dilution method: it's called One –Two D.M. (1/2)**

We transfer 1 ml of the original solution into the tube with 1ml of water, to become one part to 2 part.



Times dilution:

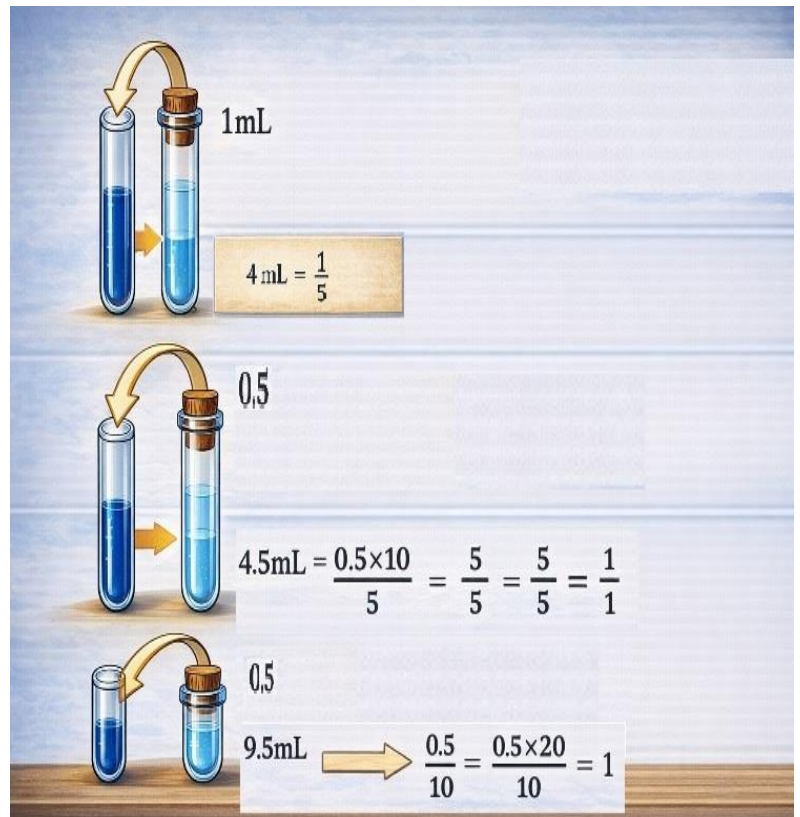
If you want to determine the No. of times dilution of a substance,

- 1) Dilution 5 times

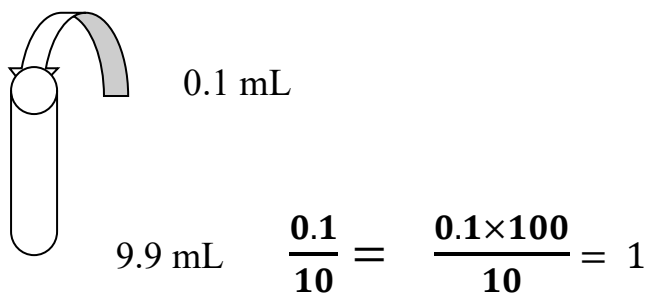
$$\frac{1 \times 5}{5} = 5$$

- 2) Dilution 10 times

- 3) Dilution 20 times



Sometime do not need the primary dilution, so depend on the double dilution of material or antibiotics Where added 0.1 mL to 9.9 mL from material diluted.



Where dilution = 100 times

- Sometimes use $C_1V_1 = C_2V_2$ to find different dilutions.
- $\text{Stock} = \frac{W.}{V.}$
 - $1\text{g} = 1000\text{mg}$
 - $1\text{mg} = 1000\text{ }\mu\text{g}$
 - $1\text{L} = 1000\text{mL}$
 - $1\text{mL} = 1000\text{ }\mu\text{L}$

Ex: prepare antibiotic with concentration $100\text{ }\mu\text{g/mL}$ from concentration $5000\text{ }\mu\text{g/mL}$ in volume 10mL .

$$C_1V_1 = C_2V_2$$

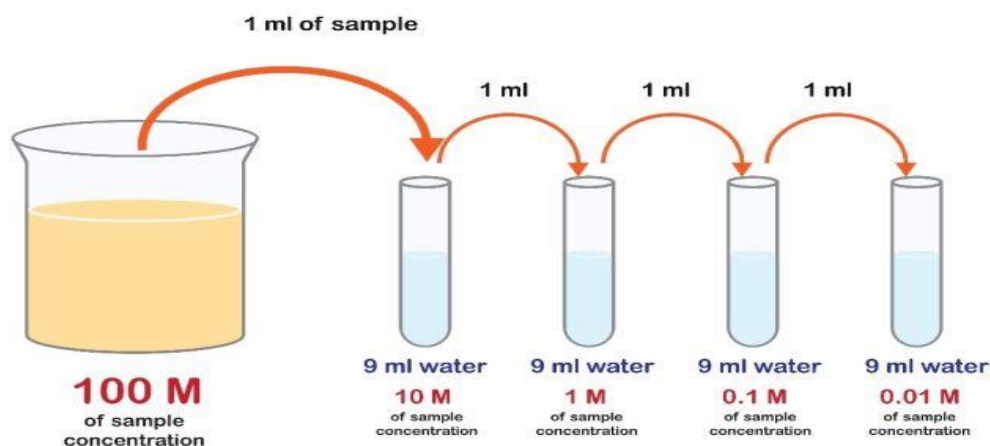
$$5000 * V_1 = 100 * 10 = V_1 = \frac{1000}{5000} = 0.2\text{ mL}$$

H.W.: Why we dilution antibiotic?

A serial dilution is a stepwise series of dilutions performed to reduce the concentration of a substance in a solution. Unlike a single dilution, where you add a large amount of solvent at once, a serial dilution uses the result of the previous step as the starting material for the next. This technique is essential in laboratories because it allows scientists to accurately reach extremely low concentrations that would be impossible to measure out directly.

How the Process Works: In a standard serial dilution, a constant volume of the sample is transferred into a series of tubes containing a fixed volume of diluent (such as water, saline, or culture media).

SERIAL DILUTION



Why do labs use 70% alcohol instead of 90%+ to kill bacteria?

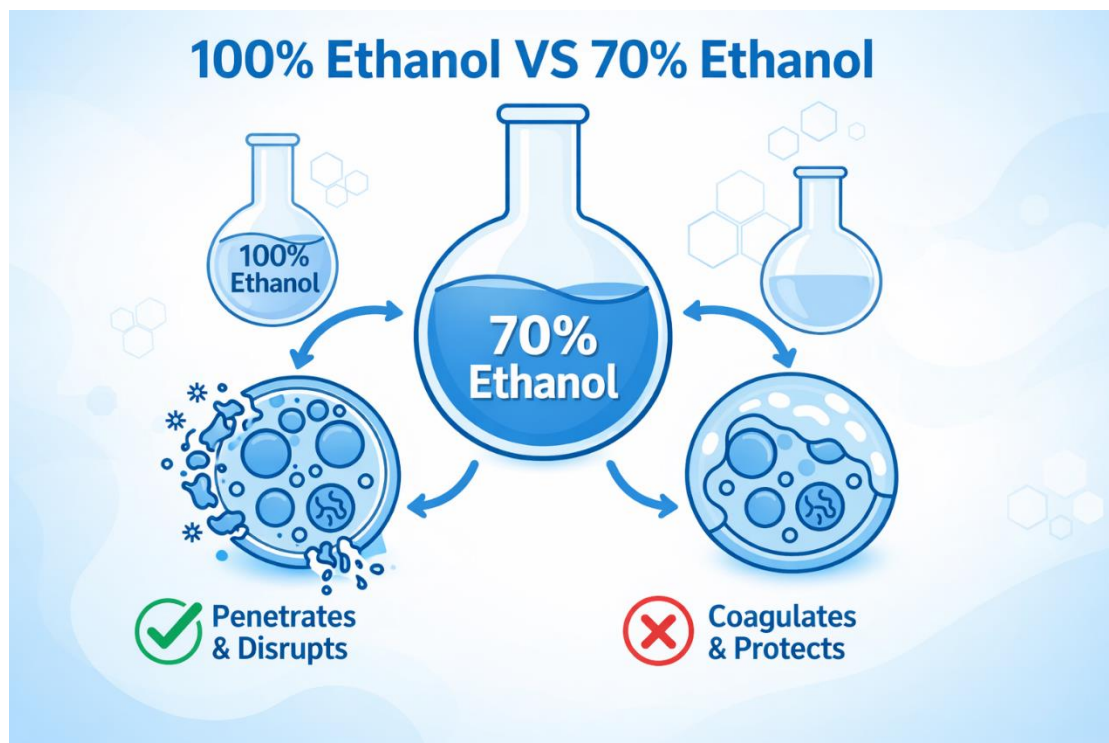
70% alcohol contains enough water to penetrate the bacterial cell wall. The water slows evaporation, giving alcohol time to work. Proteins coagulate inside the cell → bacteria die, but how?

Bacterial cells are full of proteins — enzymes, structural proteins, and membrane proteins. Proteins are made of amino acids folded into a precise structure, held together by:

1- hydrogen bonds. 2- ionic interactions. 3- hydrophobic interactions

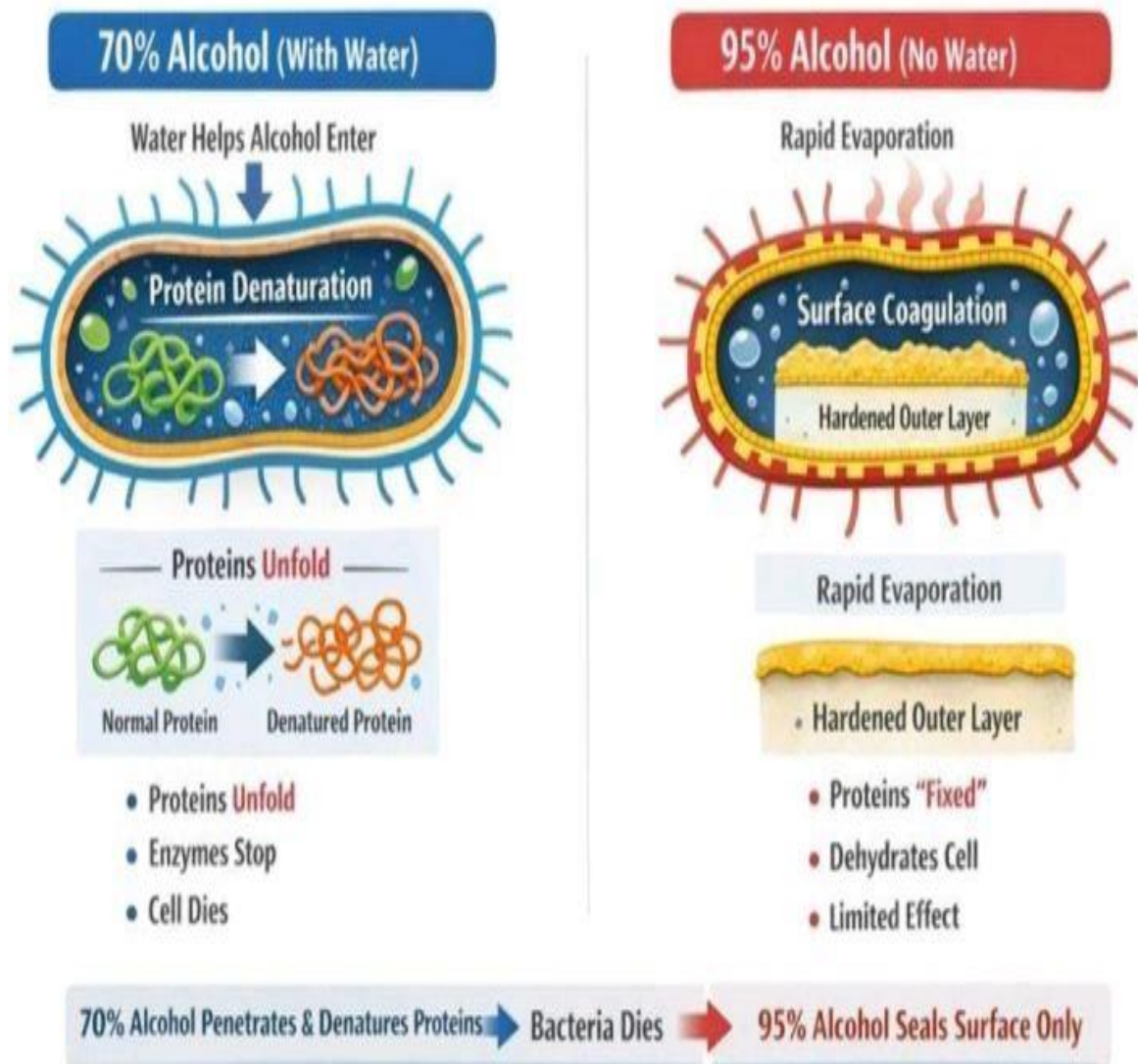
When 70% alcohol enters the cell:

- 1- Alcohol disrupts these weak interactions
- 2- Water facilitates this process by allowing alcohol to penetrate deeply
- 3- The protein unfolds (denatures) and loses its functional shape.



The cell can no longer maintain life processes. With 90–95% alcohol, rapid evaporation and surface dehydration cause proteins to coagulate only on the outside, forming a protective layer that limits penetration. A simple example of how understanding the mechanism matters more than increasing the dose.

Why 70% Alcohol Kills Bacteria Better Than 95%



H.W.:

- Prepare the serial dilution (2000, 1000, 700, 100, 25, 10, 2 $\mu\text{g/mL}$) from capsule ampicillin 250mg.
- Prepare erythromycin in concentration (600, 200, 100, 50, 10, 8, 4) $\mu\text{g/mL}$ with final volume 5mL, by using stock solution 1mg/mL, by using serial dilution method.

- Prepare erythromycin in concentration (600, 300, 100, 25, 10, 2) $\mu\text{g}\backslash\text{mL}$ with final volume 4mL, by using stock solution 1mg/mL, by using serial dilution method.
- Prepare erythromycin in concentration (800, 400, 100, 50, 25, 12.5) $\mu\text{g}\backslash\text{mL}$ with final volume 4mL, by using stock solution 1mg/mL, by using serial dilution method
- Prepare erythromycin in concentration (200, 100, 10, 8, 4,2,0.5) $\mu\text{g}\backslash\text{mL}$ with final volume by using stock solution 1mg/mL, by using serial dilution method.
- Prepare erythromycin in concentration (800, 400, 100, 50, 25, 12.5) $\mu\text{g}\backslash\text{mL}$, by using stock solution 1mg/mL, by using serial dilution method.
- Prepare erythromycin in concentration (600, 300, 100, 50, 5,1) $\mu\text{g}\backslash\text{mL}$, by using capsule 475mg, by using serial dilution method.

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Why Use Only Distilled Water in Labs?

If you often hear "**dilute with distilled water**" in analysis protocols and think tap or mineral water would do, think again! The difference between **accurate results** and **disaster** all boils down to those **extra ions** in regular water.

Why is distilled water necessary?

1. Avoid "Chemical Interference":

Tap water contains salts and ions like Calcium (Ca^{2+}), magnesium (Mg^{2+}), and chloride (Cl^-). These ions can react with reagents in the analysis, leading to inaccurate systems 'False Positive'.



2. Preserve Enzyme Activity:

In hormone or enzyme labs, heavy metals or impurities in tap water can alter the enzyme shape or **fully inhibit** its activity.

3. Maintain pH Stability:

Distilled water is nearly neutral, ensuring that the medium where the sample is diluted doesn't **alter its** chemistry or the precise pH needed for the reaction.

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4. Protect Equipment Longevity:

Using water with salts creates **scales** inside the fine tubes of Auto-analyzers, leading to clogging over time, and **calibration failure** for defacil.



Technical Tip:

In the lab, we **don't** just look for "clean" water, we want "**chemically inert**" water. Distilled water ensures the only variable in your experiment is your sample—not the **water** you used!

Advice to all lab specialists: Always check the **quality** of your distillation device, and **measure the conductivity of the water** regularly. Accuracy starts with the smallest details.

