

Antibiotic Technology Laboratory

Lab 4: Minimal Inhibitory Concentration (MIC):

The lowest concentration of antibiotic that inhibit growth of bacteria also called tube dilution method, The lowest concentration (highest dilution) of test agent preventing appearance of turbidity (growth) is considered to be the minimal / minimum inhibitory concentration (MIC). At this dilution, the test agent is bacteriostatic, and this Achieved by: **Tube dilution methods and Agar dilution method.**

Advantages of MIC:

- 1- Dose to inhibit MIC is a better predictor of appropriate drug.
- 2- Use a lower concentration which reach to the body fluid, able to use a less expensive but still effective antibody
- 3- Decrease chance of toxic effects on the patient's systems/organs.

Antibacterial susceptibility breakpoints

What are the antibacterial susceptibility breakpoints? They are a set of values through which scientists define susceptibility and resistance of bacterial strains to various antibacterial agents. These breakpoints had expressed either in concentration (mg/liter or $\mu\text{g/ml}$) or in a zone diameter (mm), and had established by many international organizations and by using different methods. Setting the antibacterial breakpoints depends usually on four different data sources that have to be taken into consideration, while the final goal of every breakpoint is classify tests results as susceptible, intermediate, or resistant. The huge variety among breakpoints have made it possible that the same pathogenic strain causing the same damage to body tissues can be identified resistant in one country and susceptible in another to the same antibacterial agent. The fact that the data sources might be collected using experimental procedures that does not resemble the in “vivo” environment questions the validity of these breakpoints. Problems with susceptibility breakpoints extended toward reliability and even toward the economic impact of wrong breakpoints.

What do breakpoints represent?

Breakpoints define the cut-off values for:

- 1- Zone diameters (mm) in disk diffusion tests
- 2- MIC values (Minimum Inhibitory Concentrations, $\mu\text{g/mL}$).

Minimum Inhibitory Concentration (MIC) Determination

To determine the **Minimum Inhibitory Concentration (MIC)**, a series of broth tubes (or wells) is prepared containing **serial dilutions of the antibiotic**. Each tube is inoculated with a **standardized bacterial inoculum**, ensuring that the **same cell density** is used across all tubes. After inoculation, the cultures are incubated under appropriate conditions, typically with a final bacterial concentration of 10^3 – 10^6 cells/mL. The MIC is defined as the lowest concentration of the antibiotic that completely inhibits visible bacterial growth after incubation and MIC helps clinicians choose the right drug and dose.

An important principle to remember is: **The more resistant an organism is, the higher its MIC value will be.**

Antimicrobial susceptibility testing methods are classified based on the **principle used to assess bacterial response to antibiotics**:

Diffusion	Dilution	Diffusion & Dilution
Stokes method	Minimum Inhibitory Concentration	E-Test method
Kirby-Bauer method	1) Broth dilution and 2) Agar Dilution	

Diffusion tells us if the organism is susceptible, while **dilution** tells us *how much antibiotic* is needed.

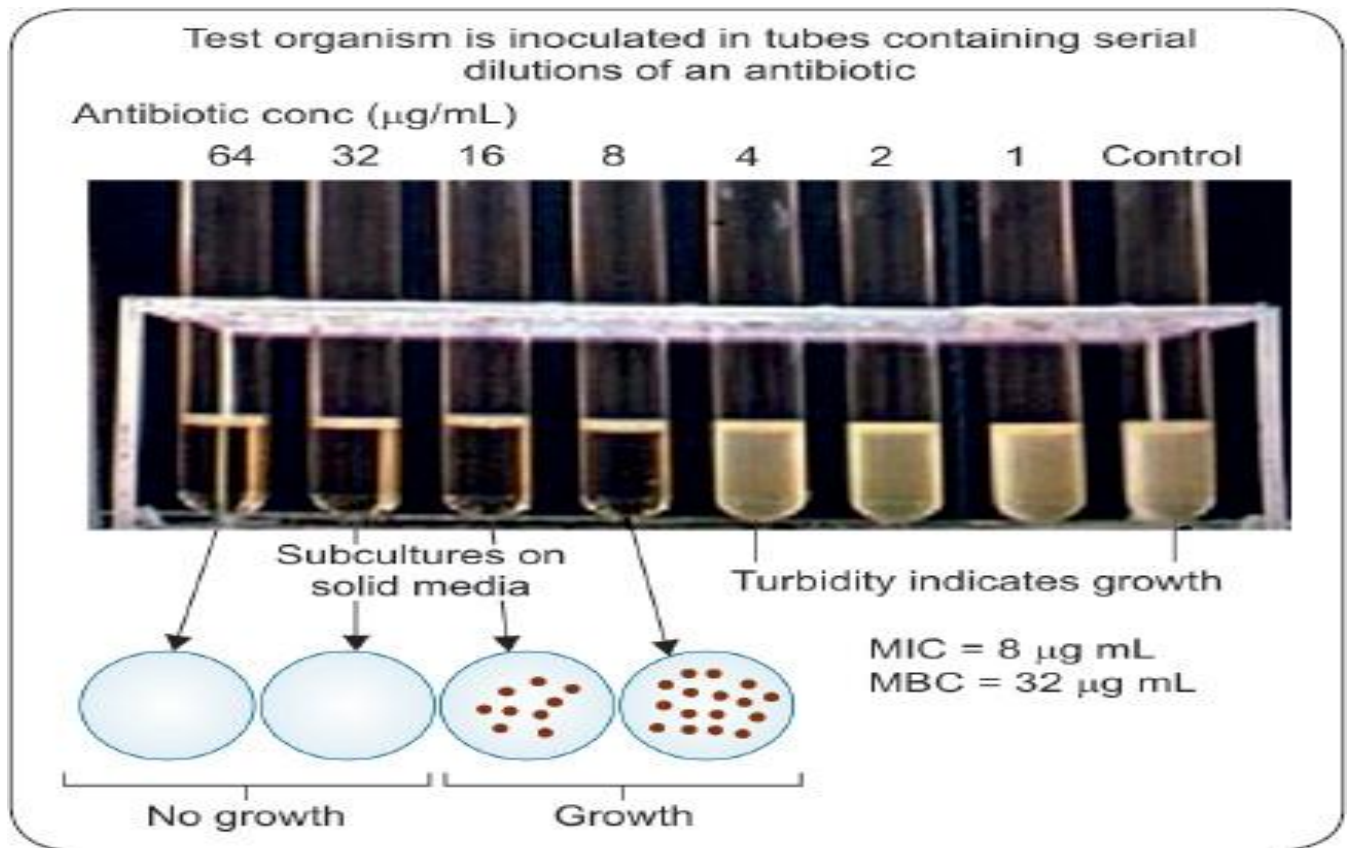
Tube dilution methods:

- 1- In this method, we use sterile Mueller-Hinton broth.

- 2- We make 2-folds dilution of antibiotics in the broth i.e. $2\mu\text{g/ml}$, $4\mu\text{g/ml}$, $8\mu\text{g/ml}$, $16\mu\text{g/ml}$ and so on.
- 3- Then we add broth culture (0.1ml) of test organism to the prepared dilutions.

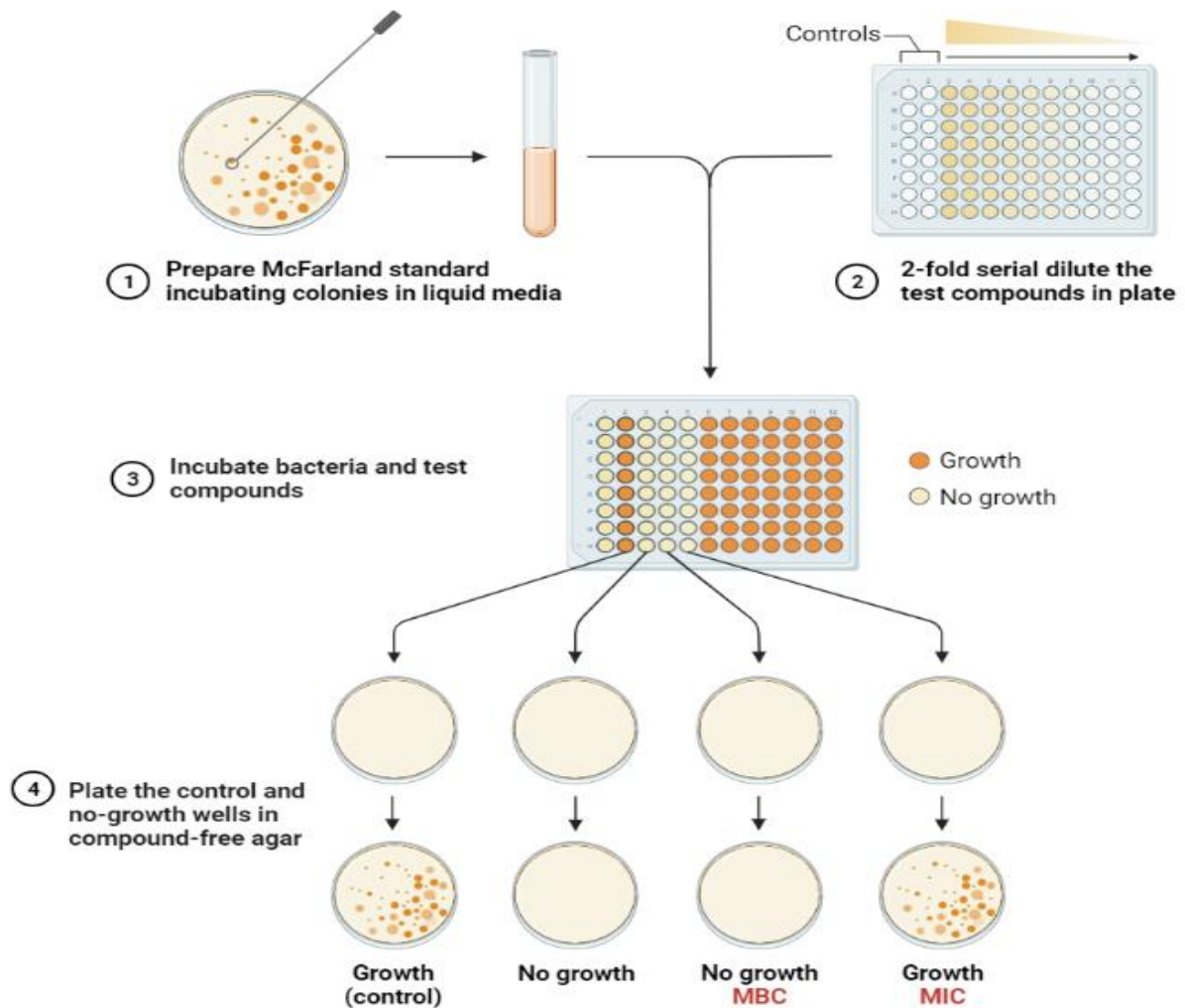
Procedure:

- 1- Arrange sterile test tubes in a rack and dispense 1 ml of sterile Müller-Hinton broth into each tube.
- 2- Prepare a stock solution of the antimicrobial agent and diluted according to your serial dilution to your test. The value of MIC according to CLSI is: (0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024).
- 3- Start to do the serial dilution according to your value. Make two-fold dilution of the antimicrobial agent by transferring 1 ml of the solution from the solution from the first tube to the second one.
- 4- Continue to make serial dilution till the entire range is covered. The last tube concentration is $0.5\mu\text{g/ml}$ and let two tube one of them without add anything as it acts as growth control (positive control). In addition, the second just broth and antibiotic without inoculated by bacteria (negative control).
- 5- Adjust the turbidity of bacterial suspension overnight growth by 0.5 McFarland standard.
- 6- Unify the sizes in all the tube according to the less size of tubes.
- 7- Add 0.1 mL of this suspension to each dilution and control tube.
- 8- Incubate the tubes in 16-18hr at 37°C .
- 9- Results by compare with the control tube read all tubes for visible growth record the result. The lowest concentration with no visible growth is the MIC for the test organism.



Homework:

- 1- Why we do serial dilution of antibiotics in MIC??
- 2- Why we add the same size of bacteria to all tubes??



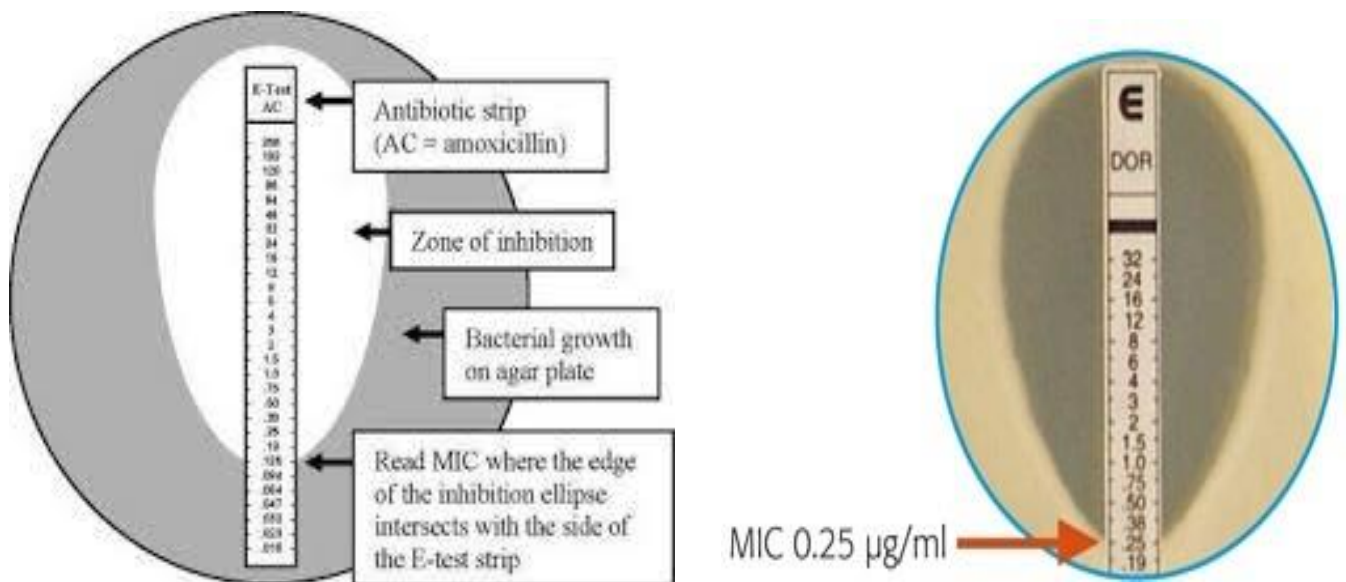
Results

MIC: Minimum inhibitory concentration. Bacteria cannot grow when incubated with test compound but can be rescued when plating in compound-free agar.
MBC: Minimum bactericidal concentration. Bacteria cannot grow when incubated with test compound and cannot be rescued when plating in compound-free agar.

Epsilometer test or E-test or MIC Test Strip: It is a quantitative assay for determining the MIC of antimicrobial agents against microorganisms and for detecting the resistance mechanisms. Plastic strips with a predefined gradient of one antibiotic. Paper strips with special features that are impregnated with a predefined concentration gradient of antibiotic. On one side of the strip is indicated a MIC scale in $\mu\text{g/ml}$ and a code that identifies the antimicrobial agent.

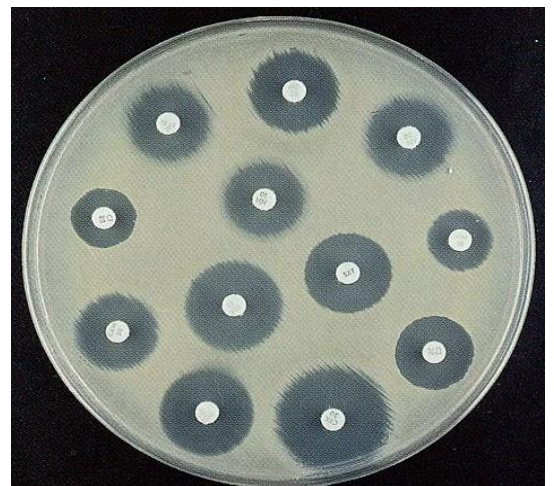
E-test is based on arraying a concentration gradient of each antibiotic on a polymer strip. **Concentration values** are marked on the other side of the strip so that one can easily locate corresponding concentrations

The exponential gradient of antimicrobial agent is immediately transferred to the agar matrix. After 16-18 hours incubation or longer, a symmetrical inhibition ellipse centered along the strip is formed. The MIC is read directly from the scale in terms of $\mu\text{g/ml}$ at the point where the edge of the inhibition ellipse intersects the MIC Test Strip, and it's have Wide range of antibiotics, Easy to use, Storage at -20°C , Short shelf life, expensive, Simple, Active.



Reading Plates and Interpreting Results in sensitivity test:

- Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate.
- Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.
- After 16 to 18 hours of incubation, each plate is examined.



- If individual colonies are apparent, the inoculum was too light and the test must be repeated.
- The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc.
- Zones are measured to the nearest whole millimeter, using a ruler, which is held on the back of the inverted Petri plate.
- The Petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light.
- If blood was added to the agar base (as with streptococci), the zones are measured from the upper surface of the agar illuminated with reflected light, with the cover removed.
- The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye.
- Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored.
- However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified, and retested.
- Strains of *Proteus spp.* may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus spp.*, the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored.
- When using blood-supplemented medium for testing streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis.

Not every antibiotic reported as “Sensitive” can be used as a treatment

Many people fall into this common mistake When a culture report shows “Sensitive”, it is often assumed that the antibiotic is automatically suitable

The truth is that three very important factors must be considered before choosing an antibiotic:

[1] Site of infection, not every antibiotic reaches all tissues effectively.

For example:

- Some antibiotics achieve excellent levels in the blood
- But are very weak in the urine or the lungs

[2] Drug concentration (MIC): An antibiotic may appear sensitive on paper, but the dose required to reach an effective concentration may be toxic to the patient

[3] Patient condition like: Kidney function, Liver function, Age, Immune status

Important example: Gentamicin may be reported as *Sensitive*, but it should not be used in a patient with renal failure

Therefore: Laboratory results must always be interpreted in the context of the clinical condition

The final decision is clinical, not purely numerical

The laboratory is not just about tests... the laboratory is a responsibility for life.



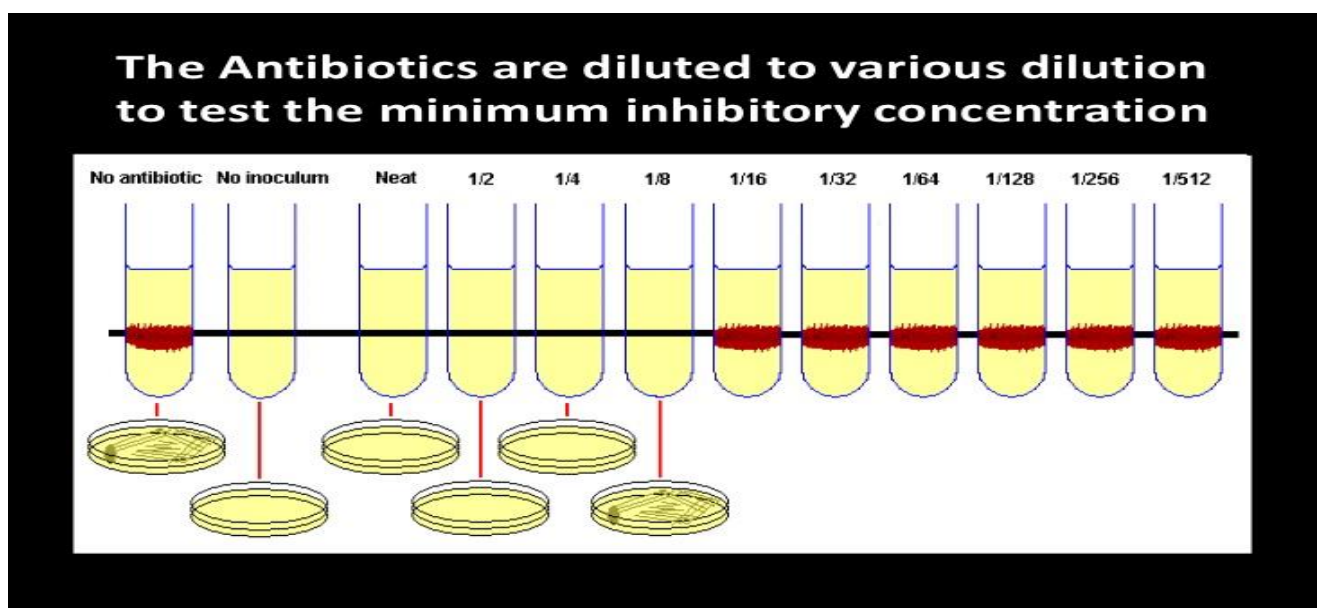
Minimum Bactericidal Concentration (MBC):

The minimal bactericidal concentration (MBC) or the minimum lethal concentration (MLC) can be determined by sub culturing all tubes showing no visible turbidity, of an antibacterial, which is defined as the maximum dilution of the product that will kill a test, organism can be determined by sub culturing last clear MIC tube onto growth medium and examining for bacterial growth. Serial dilutions are made of the products in bacterial growth media. The tube with the highest dilution that fails to yield growth on the subculture plate contains the MBC of antibiotic for the test strain. The MBC was measured by inoculating the broths used for MIC determinations onto drug-free medium. The MBC is the first dilution at which no growth was observed.

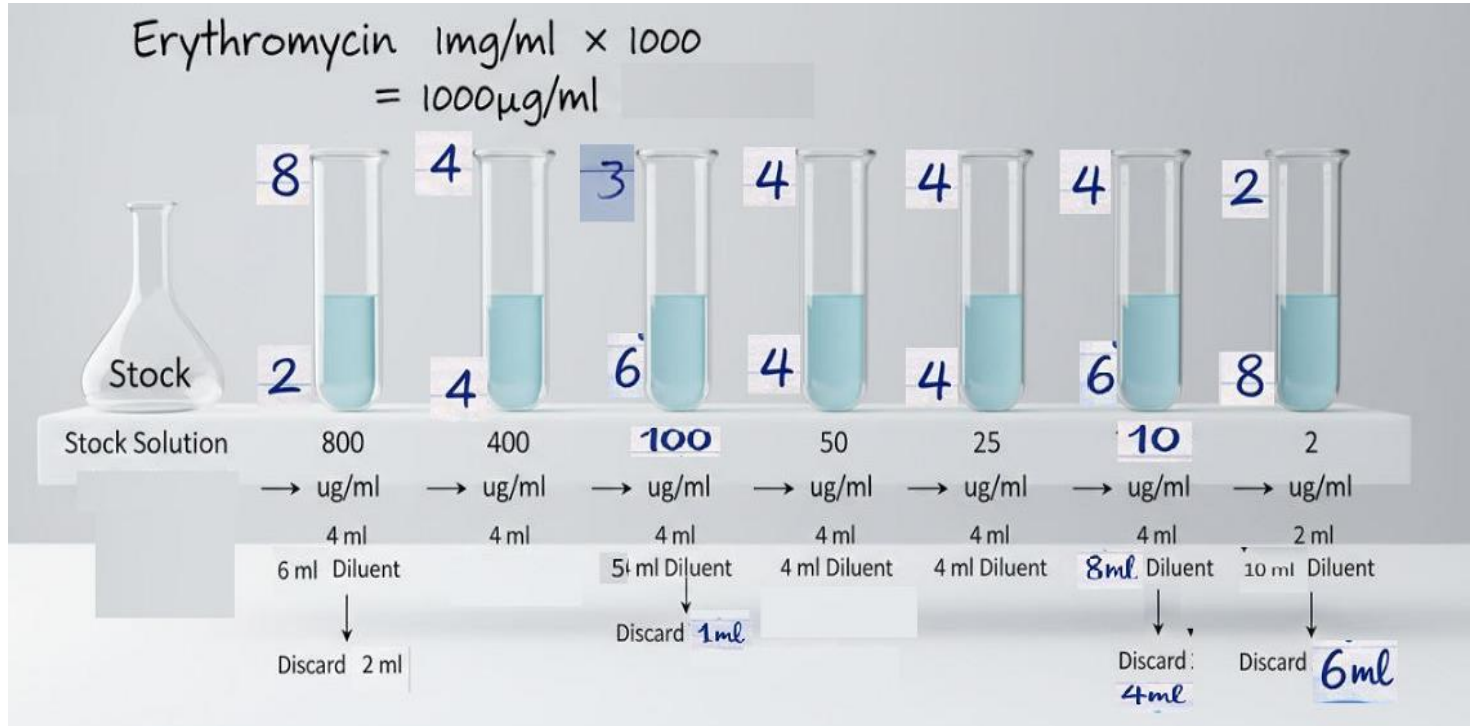
Procedure:

- 1- The same steps of determination MIC, after we got the result follow this
- 2- We culture all clear tubes in MIC and start from the first dilution at which no growth was observed, the culture by taken (0.1mL) from all clear tubes and streaking on MHA and incubate 16-18 hrs.

After incubation period, we observed the plate, which do not contain growth, so this plate or concentration is MBC, which kill growth of bacteria, the MIC is much lower than the MBC or maybe equal this MBC value.



- ❖ Prepare erythromycin in concentration (800, 400, 100, 50, 25, 12.5) $\mu\text{g/mL}$ with final volume 4mL, by using stock solution 1mg/mL, by using serial dilution method



- ❖ Prepare erythromycin in concentration (600, 300, 100, 25, 10, 2) $\mu\text{g/mL}$ with final volume 4mL, by using stock solution 1mg/mL, by using serial dilution method.
- ❖ 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 (200, 100, 10, 8, 4, 2, 0.5) $\mu\text{g/mL}$ with final volume 3mL, by using stock solution 1mg/mL, by using serial dilution method.