

LAB.9. Technique of antibiotic sensitivity

2- DILUTION TEST

- MINIMAL INHIBITORY CONCENTRATION TEST

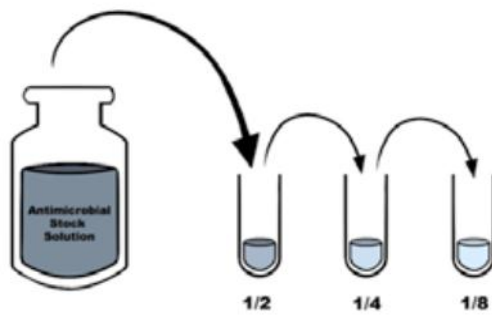
The **minimal inhibitory concentration (MIC)** is the lowest concentration of an antibiotic that inhibits the growth of a microorganism.

Minimum Bactericidal Concentration (MBC): the lowest concentration of an antimicrobial agent required to achieve bactericidal (killing bacteria).

Quantitative testing uses a dilution technique in which tubes containing serial dilutions of an antibiotic are inoculated with the organism whose sensitivity to that antibiotic is to be tested. The tubes are incubated and later observed to determine the minimal inhibitory concentration (MIC) of the antibiotic necessary to prevent bacterial growth .To provide effective antimicrobial therapy, the clinically obtainable antibiotic concentration in body fluids should be greater than the MIC. Quantitative susceptibility testing may be necessary for patients who either fail to respond to antimicrobial therapy or who relapse during therapy. In some clinical cases, the minimal bactericidal concentration (MBC) may need to be determined. This is the lowest concentration of antibiotic that kills 100% of the bacteria, rather than simply inhibiting growth.

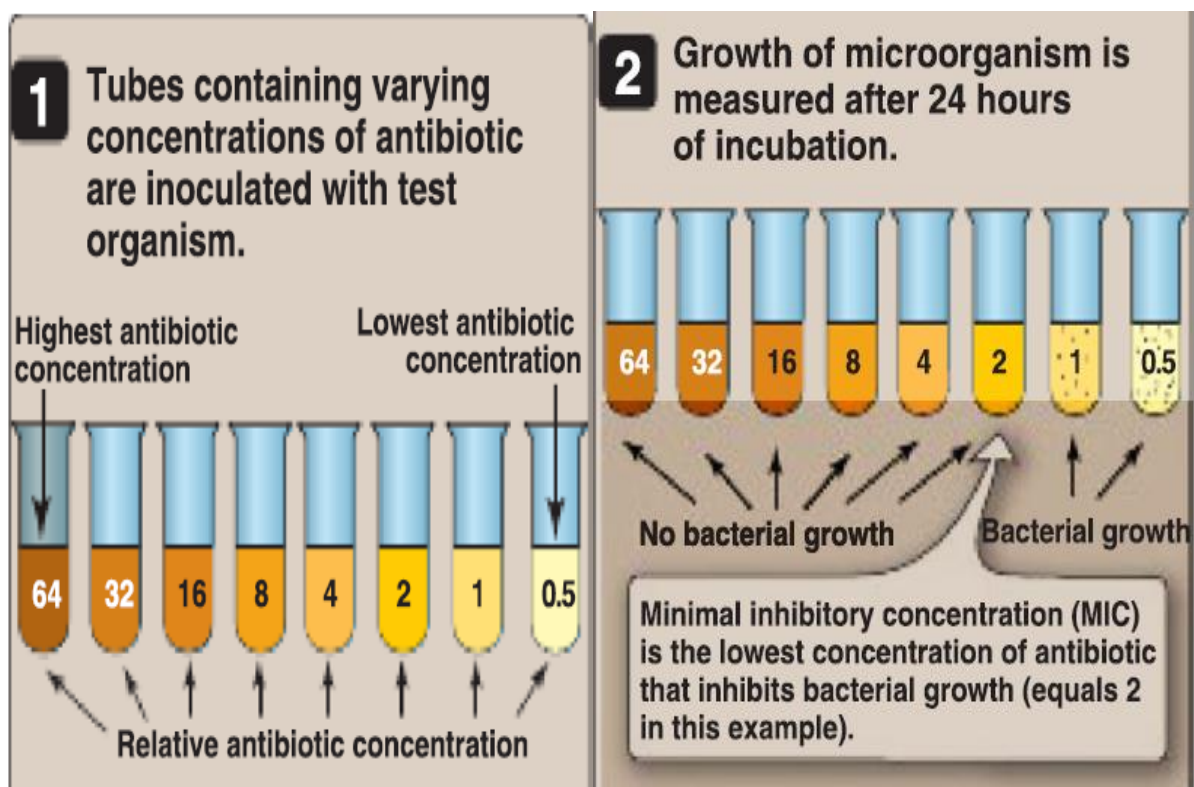
PREPARATION OF ANTIMICROBIAL AGENT STOCK SOLUTION

1. Weigh appropriate amount of the powdered antimicrobial agent.
2. Dissolve antimicrobial agent powder in solvent as indicated by manufacturer to make a concentration of at least 1,000 µg/ml or at least 10 times the highest concentration to be tested.
3. Dispense small volumes of the sterile stock solutions into sterile glass, polypropylene, polystyrene, or polyethylene vials. Carefully seal and store preferably at -60 °C or below.
4. Prepare intermediate (10x) antimicrobial agent solutions by making successive (two-fold) 1:2, 1:4, and 1:8 dilutions into sterile diluent.



INOCULUM PREPARATION

1. Grab 3-5 well-isolated colonies from a pure overnight bacterial culture and subculture it to a tube containing 4-5 ml of a suitable broth medium such as tryptic soy broth.
2. Incubate the broth culture at $35 \pm 2^\circ\text{C}$ until it achieves or exceeds a McFarland turbidity of 0.5.
3. Adjust turbidity of the inoculum with sterile saline or broth to achieve a turbidity of a 0.5 McFarland standard. Use a photometric device or adequate light to compare the inoculum tube and the 0.5 McFarland standard against a paper with white background and contrasting black lines.



Determination of minimal inhibitory concentration (MIC) of an antibiotic.