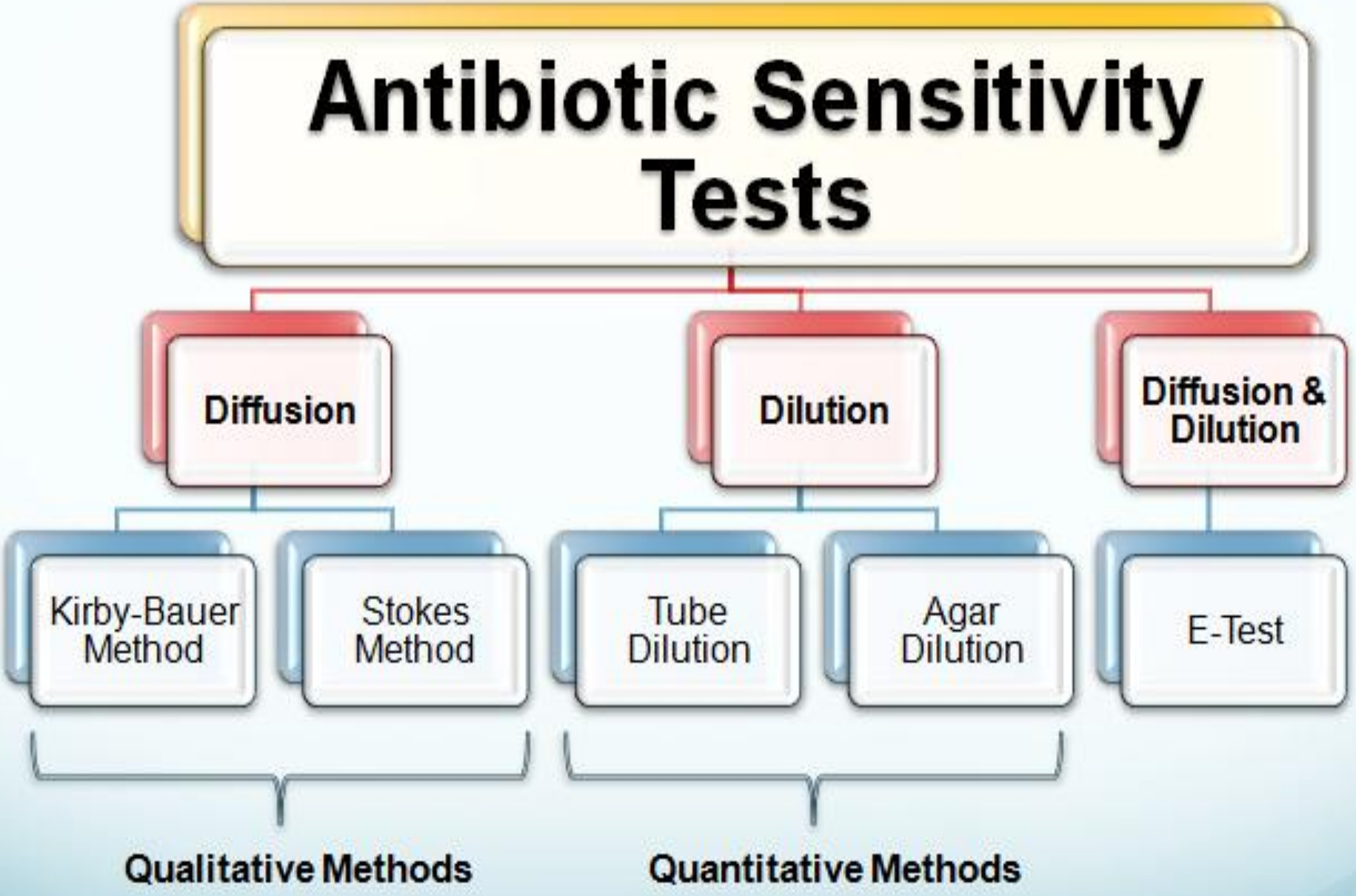
Antibiotic Susceptibility Tests

Antibiotics are:

- Natural or synthetic products that are used to kill or stop the growth of Bacteria :
- bacteriostatic - stop growth (don't kill)
- bactericidal – kill

- A test done to check the effectiveness of a drug against a bacterium and to select the best drug that acts against the bacterium.

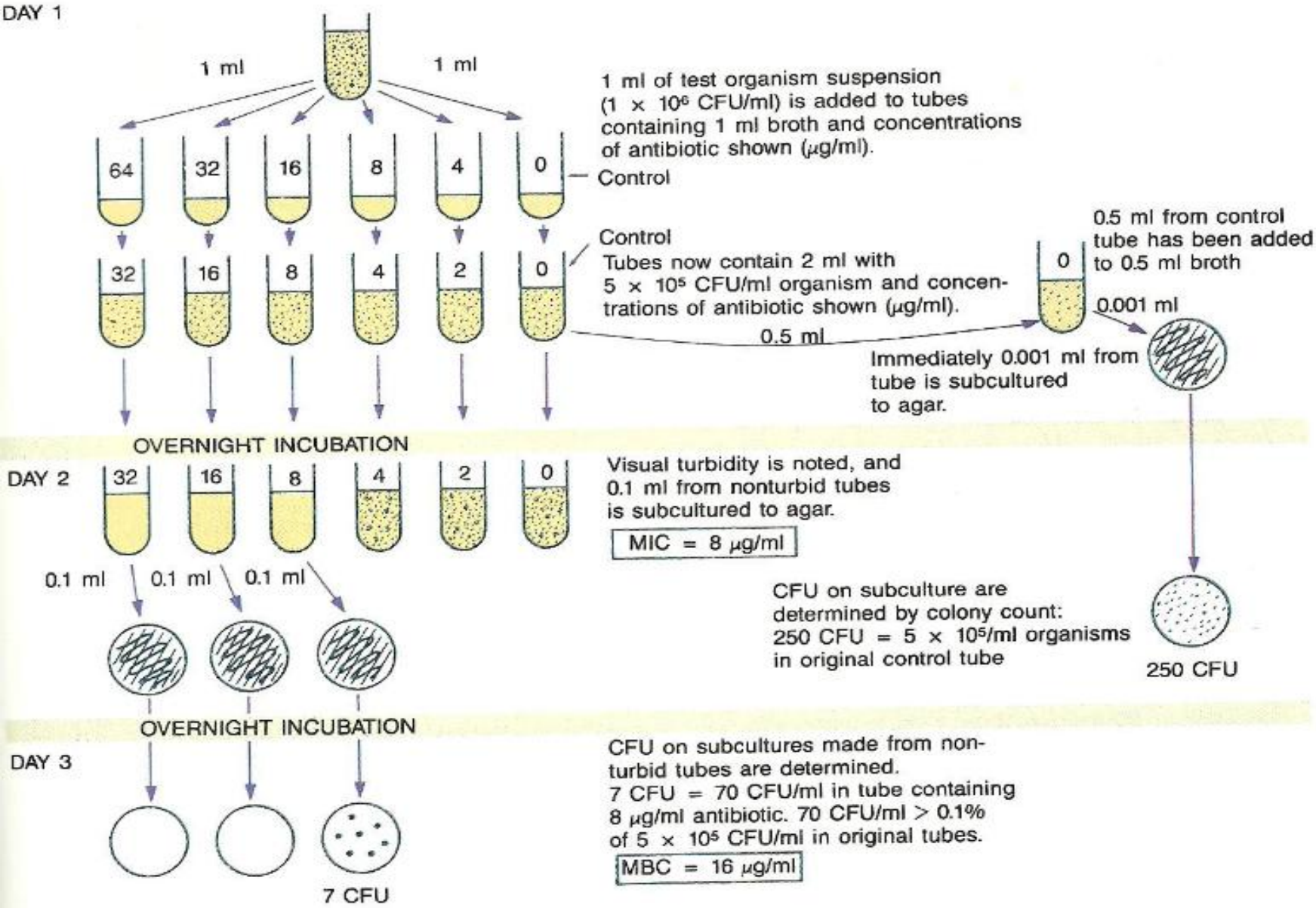

Antibiotic Sensitivity Tests
Diffusion
Dilution
Diffusion & Dilution
Kirby-Bauer Method
Stokes Method
Tube Dilution
Agar Dilution
E-Test
Qualitative Methods
Quantitative Methods

Tube dilution tests

A series of culture tubes are prepared, each containing a liquid medium and a different concentration of a chemotherapeutic agent. The tubes are then inoculated with the test organism and incubated for 16-20 hours at 35C. After incubation, the tubes are examined for turbidity (growth).

Minimum Inhibitory Concentration (MIC):
Is the lowest concentration of chemotherapeutic agent capable of preventing growth of the test organism.

Minimum Bactericidal Concentration (MBC):
Is the lowest concentration of the chemotherapeutic agent that results in no growth (turbidity) of the subcultures.


DAY 1
1 ml
1 ml
64
32
16
8
4
0
Control
1 ml of test organism suspension (1 × 10⁶ CFU/ml) is added to tubes containing 1 ml broth and concentrations of antibiotic shown (μg/ml).
32
16
8
4
2
0
Control
Tubes now contain 2 ml with 5 × 10⁵ CFU/ml organism and concentrations of antibiotic shown (μg/ml).
0.5 ml
0
0.5 ml from control tube has been added to 0.5 ml broth
0.001 ml
Immediately 0.001 ml from tube is subcultured to agar.
OVERNIGHT INCUBATION
DAY 2
32
16
8
4
2
0
Visual turbidity is noted, and 0.1 ml from nonturbid tubes is subcultured to agar.
MIC = 8 μg/ml
0.1 ml
0.1 ml
0.1 ml
CFU on subculture are determined by colony count: 250 CFU = 5 × 10⁵/ml organisms in original control tube
250 CFU
OVERNIGHT INCUBATION
DAY 3
CFU on subcultures made from nonturbid tubes are determined.
7 CFU = 70 CFU/ml in tube containing 8 μg/ml antibiotic. 70 CFU/ml > 0.1% of 5 × 10⁵ CFU/ml in original tubes.
MBC = 16 μg/ml
7 CFU

2. The agar diffusion test (Kirby- Bauer test)

This is one of the more commonly used methods of antimicrobial susceptibility testing. In this test, small filter paper disks (6 mm) impregnated with a standard amount of antibiotic are placed onto an agar plate to which bacteria have been swabbed.

The plates are incubated overnight, and the zone of inhibition of bacterial growth is used as a measure of susceptibility. Large zones of inhibition indicate that the organism is susceptible , while small or no zone of inhibition indicate resistance.

An interpretation of intermediate is given for zones which fall between the accepted cutoffs for the other interpretations. Many factors are involved in sensitivity disk testing and must be carefully controlled .these include size of inoculum, distribution of inoculum, incubation period, depth of the agar, diffusion rate of antibiotic, concentration of antibiotic in the disk, and growth rate of the bacterium .


MATERIALS
Mueller-Hinton Agar
Antibiotic Disks
Turbidity Standard
Swabs

Material:

-Mueller-Hinton agar plates

-Antibiotics disks

-sterile swabs

-(4- to 6-hour) broth cultures of *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae.*

-incubator

-forceps

-metric rulers

-(70%) ethyl alcohol and beakers

-Bunsen burner

Method:

First Period

1) inoculate the surface of four Mueller-Hinton plates with *S. aureus, E. coli, P. aeruginosa,* and *K. pneumoniae,* respectively.Use a separate, sterile cotton swab for each bacterium. The swab is immersed in the culture tube, and the excess culture is squeezed on the inner side of the test tube.

2) The swab is then taken and streaked on the surface of the Mueller-Hinton plate three times, rotating the plate 60°after each streaking. Finally, run the swab around the edge of the agar.

3) Allow the culture to dry on the plate for 5 to 10 minutes at room temperature with the top in place.

3) Dispense the antibiotics onto the plate with alcohol-flamed Forceps.

4) gently pressing the disk with alcohol-flamed Forceps, make sure that contact is made between the antibiotic disk and the culture.

5) Incubate the plates for 16 to 18 hours at 35°C.

Second Period

1) The diameter of the **inhibition zone** around the disk is measured to the nearest millimeter for each of the antibiotics tested, (by using metric rulers)

2) Determine whether each organism is **susceptible, moderately susceptible, intermediate, or resistant to each chemotherapeutic agent** using the standardized table .

3) record your results for every antibiotic on the table .

Thank you