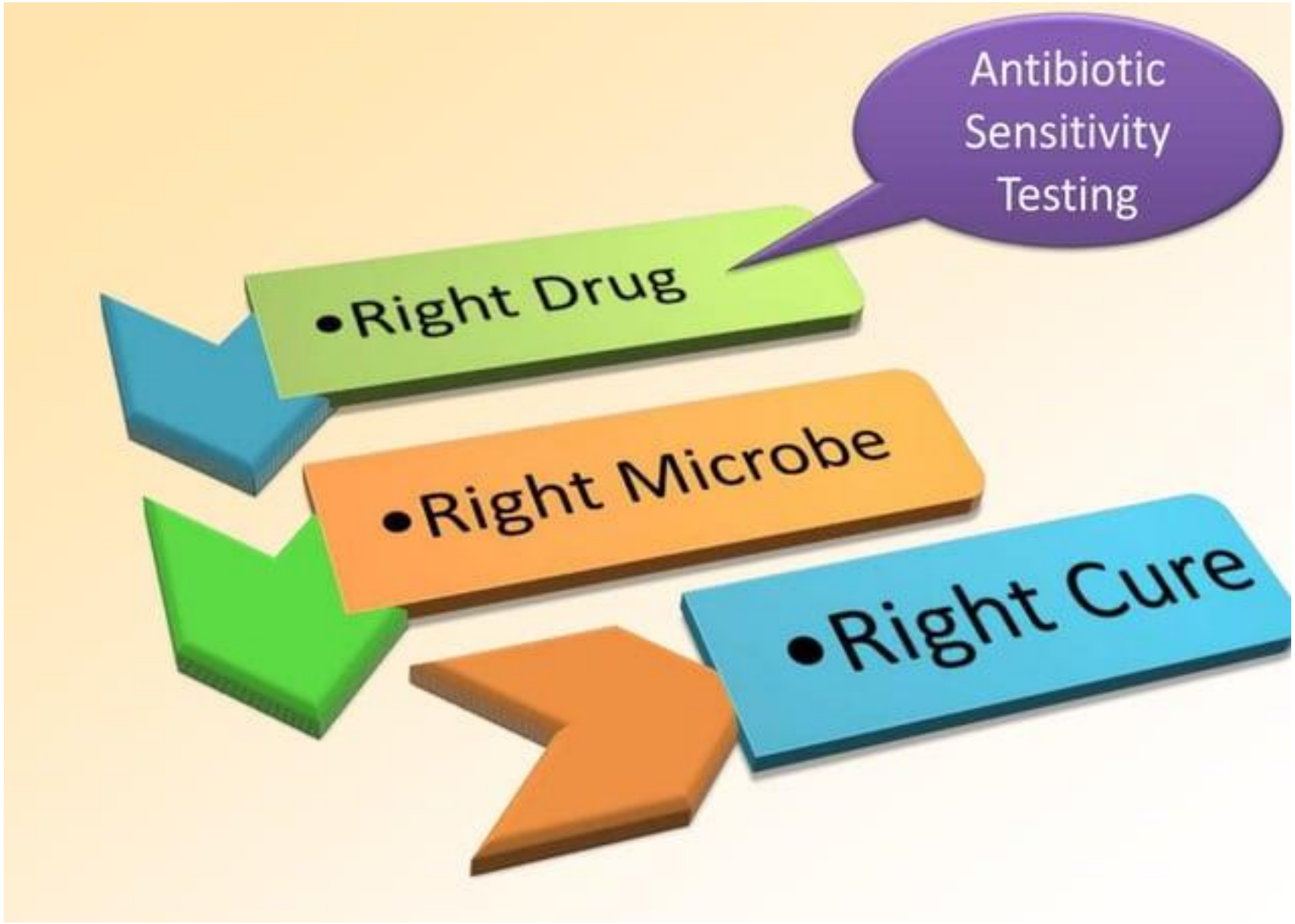
Lab – 6

Antibiotic sensitivity tests

Department of Microbiology
College of Medicine

Antibiotic sensitivity test

- 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 Testing
•Right Drug
•Right Microbe
•Right Cure

Testing for antibiotic sensitivity is often done by:

1. **Disk diffusion methods (Kirby-Bauer method).**
2. **Tube dilution methods for Minimum Inhibitory Concentration (MIC) determination.**
3. **Diffusion and Dilution Method [Epsilometer test (E test)].**

1. Disk diffusion methods

Or Kirby-Bauer method or disk diffusion test

Disk diffusion methods

- The culture used for antibiotic sensitivity testing called the **Muller Hinton Agar.**
- Small filter paper disks containing a defined amount antibiotics are placed onto a plate upon which bacteria are growing.
- The antibiotic diffuses from the disk into the agar.
- If the bacteria are sensitive to the antibiotic, a clear ring, or **zone of inhibition**, is seen around the disk indicating poor growth.
- Using special comparators that interpret the diameter of the zones of inhibition, consequently the organism can be described as resistant, intermediate, or sensitive.

Procedure

1. Firstly, the Muller Hinton Agar (MHA) plates and Muller Hinton Broth are prepared.

2. The bacteria to be tested are then introduced into these broths by inoculating a loop and incubated at 37 degrees Celsius for 24 hours.

3. Label the MHA plates (petri-dish) precisely with the organism name and adjust the turbidity of bacterial suspension. Using a sterile cotton bud, swab the surface of the plate completely with continuous rotation to create a uniform layer of bacteria.

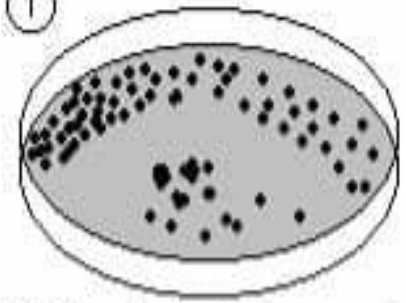
4. Flame sterilize the forceps with alcohol before picking up the discs.

5. Now, place the disc carefully over the seeded plate and tap lightly.

6. Incubate the plates at 37-degree Celsius for 24 hours.

7. After incubation, the zone of inhibition is measured and compared with CLSI guidelines to evaluate the outcomes.

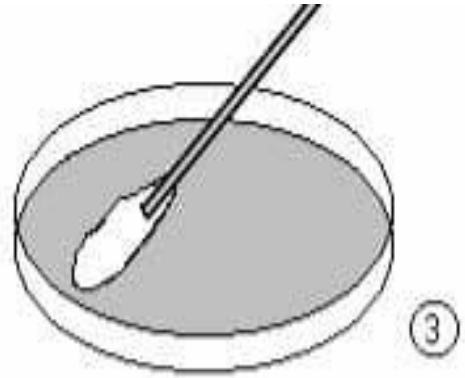
Procedure for disk diffusion testing



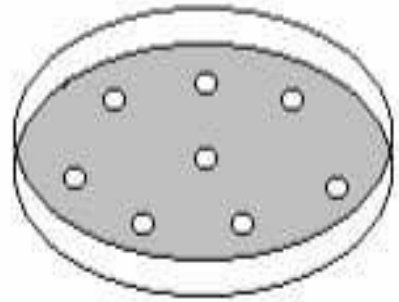
2-3 identical colonies are picked from the plate and transferred to the broth

The tube is incubated for the bacteria to grow.

The inoculum density is standardized using McFarland standard

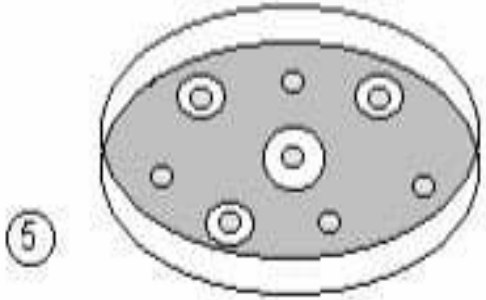


A cotton swab dipped in the inoculum suspension is swabbed over the entire surface of agar to give a lawn culture.



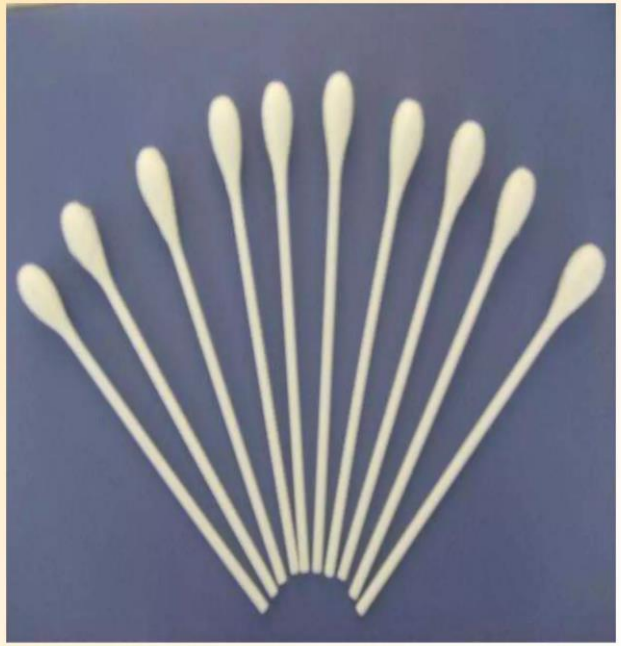
Filter paper disks containing known antibiotic in known concentration is placed

Plate incubated at 37C overnight



Zone diameter around the disk are measured and result read from Kirby Bauer chart

Cotton Swabs



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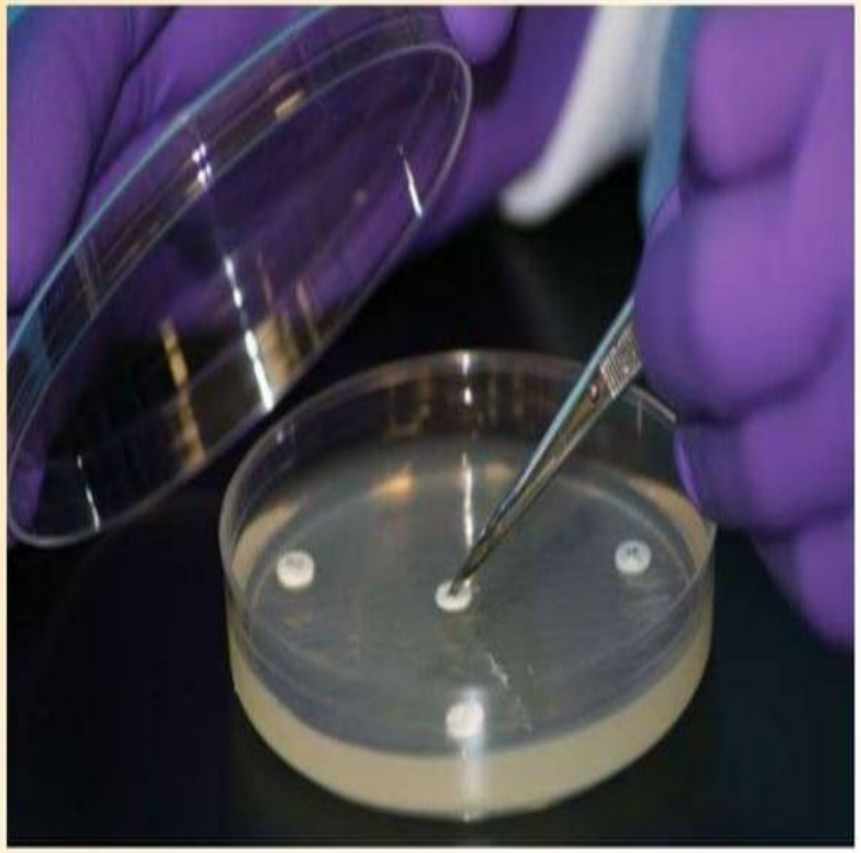
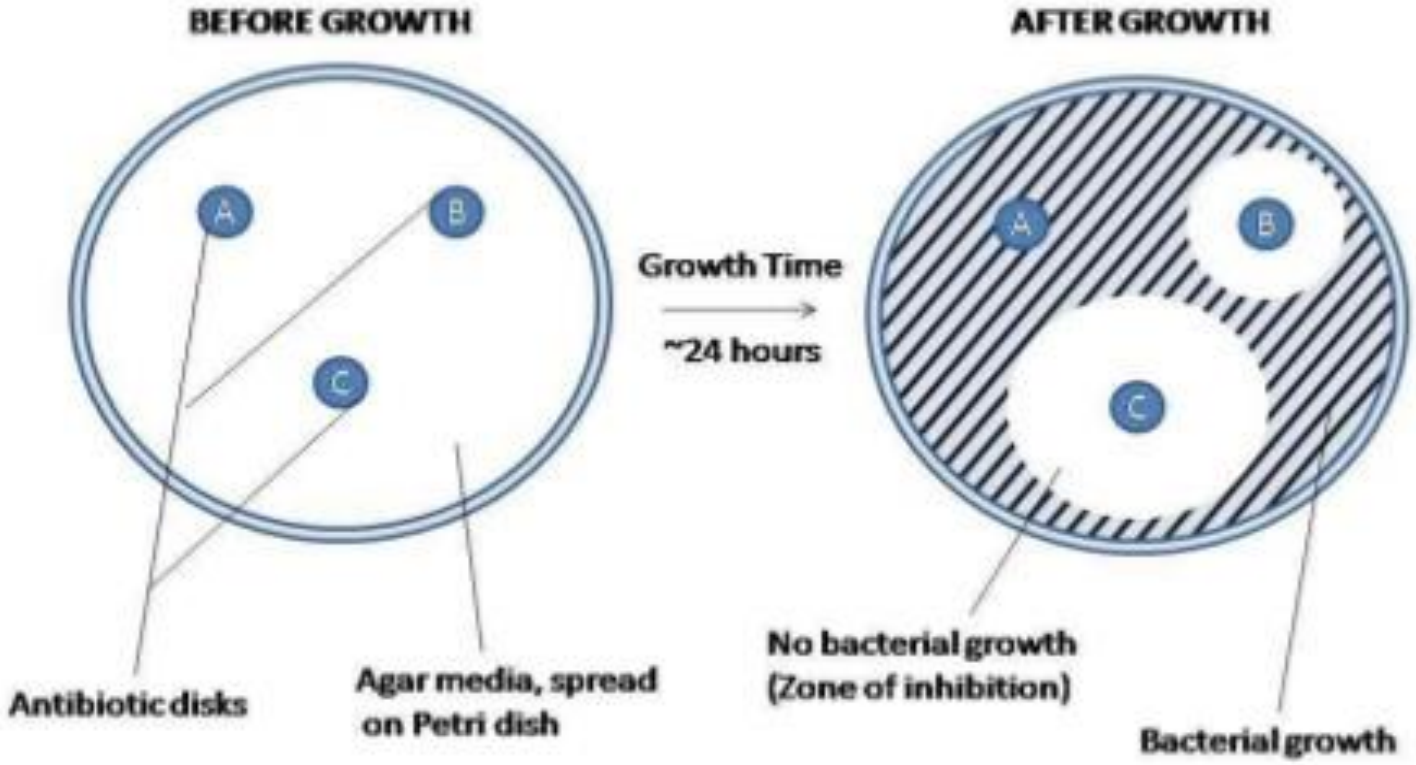
Antibiotic Disks

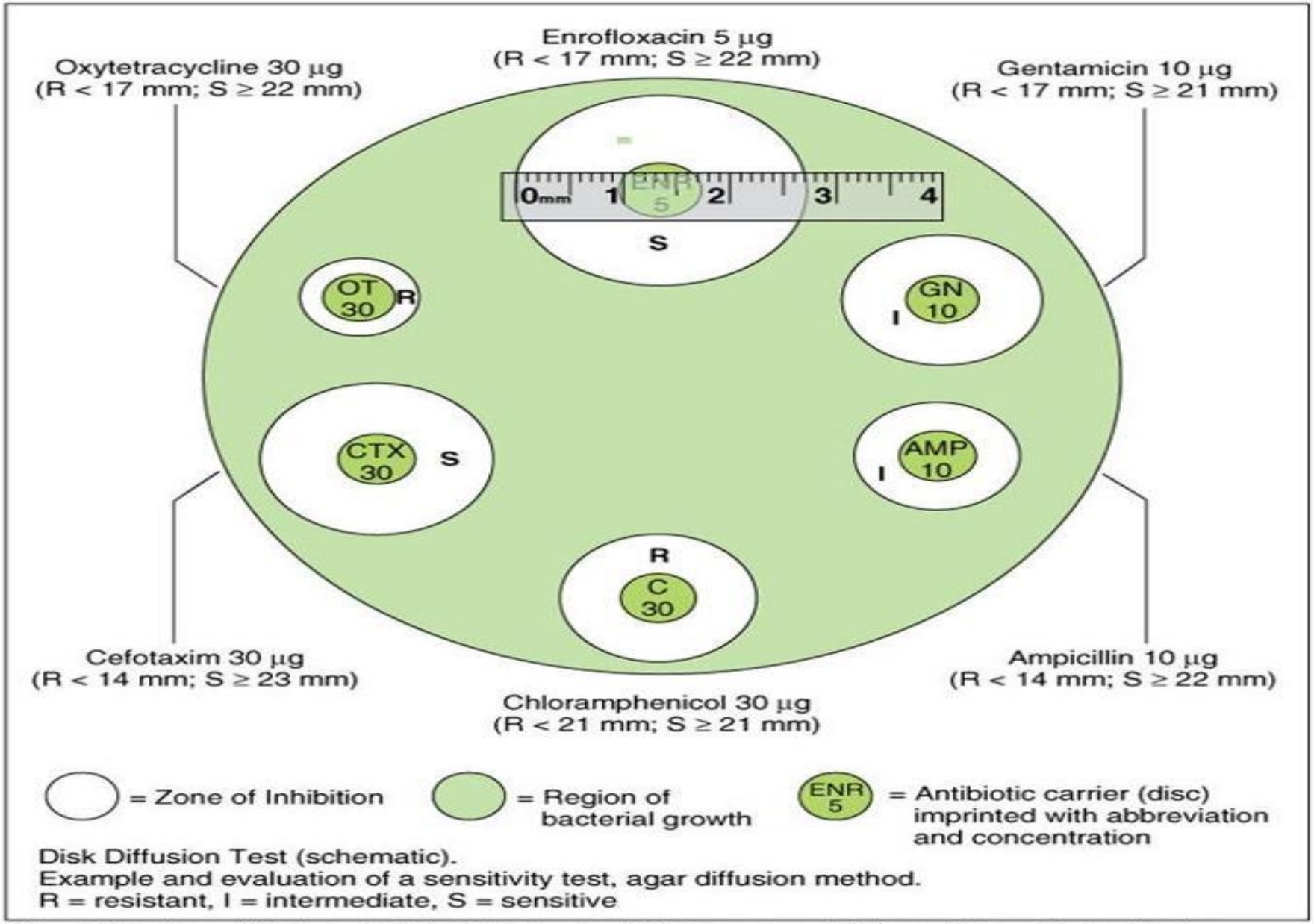


Result


Antibiotic


BEFORE GROWTH
AFTER GROWTH
A
B
C
Growth Time
~24 hours
Antibiotic disks
Agar media, spread on Petri dish
No bacterial growth (Zone of inhibition)
Bacterial growth

Kirby-Bauer Disk Diffusion Test*



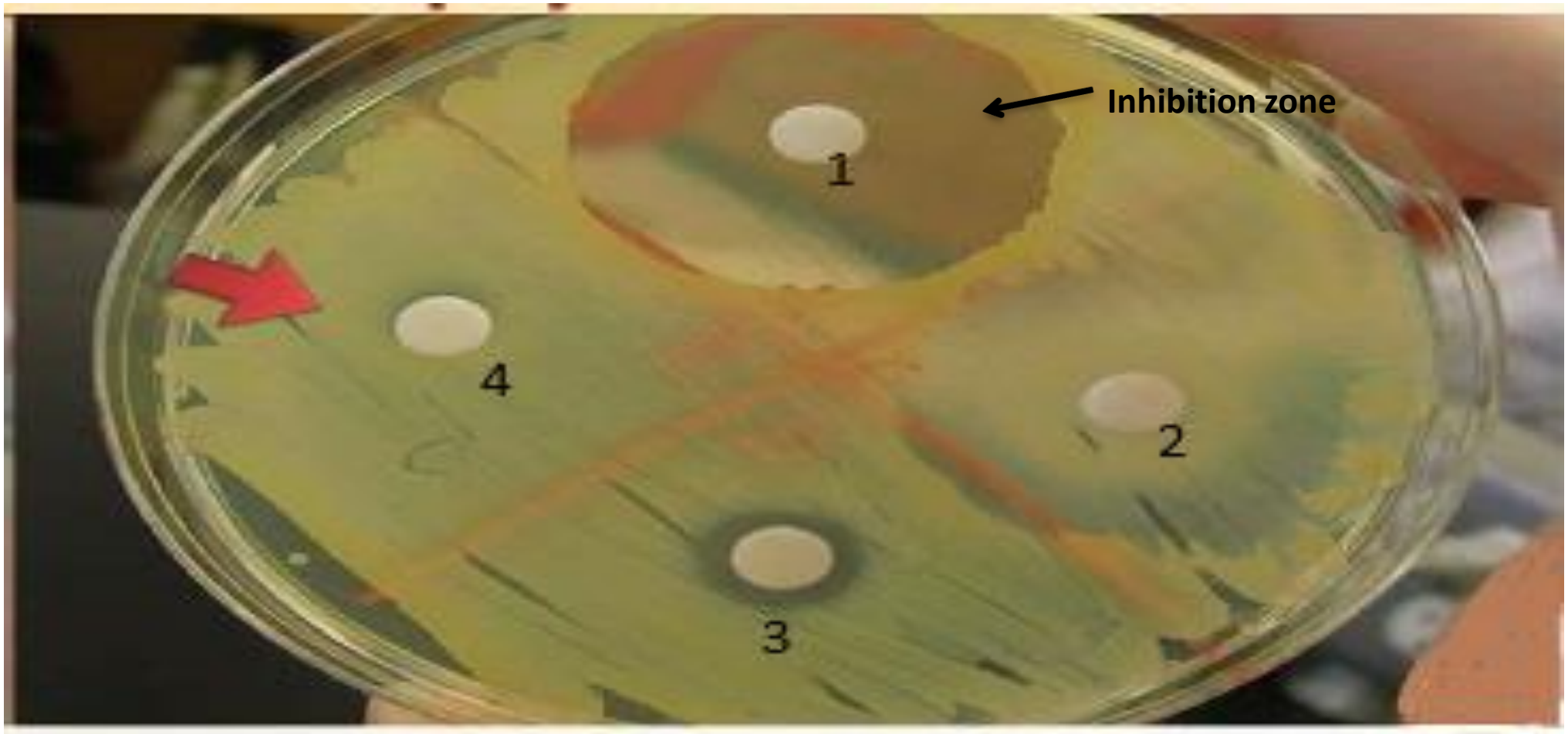
Disk Diffusion Test (schematic).
Example and evaluation of a sensitivity test, agar diffusion method.
R = resistant, I = intermediate, S = sensitive

(a)

*R and S values differ from Table 12.7 due to differing concentrations of the antimicrobials.

Using a ruler measure the diameter of any zones of inhibition and record your results, the results must be compared with values listed in standard charts as shown in the interpretative chart below:

Antibiotic	Disk concentration	Diameter of zone of inhibition		
		Resistant	Intermediate	Susceptible
ampicillin	10 microgram	11 or less	12-13	14 or more
cephalothin	30 microgram	14 or less	15-17	18 or more
chloramphenicol	30 microgram	12 or less	13-17	18 or more
gentamicin	10 microgram	12 or less	13-14	15 or more
penicillin	10 U	20 or less	21-28	29 or more
Polymyxin B	300 U	8 or less	8-11	12 or more
sulphonamide	300 microgram	12 or less	13-16	17 or more
tetracycline	30 microgram	14 or less	15-18	19 or more



- Bacteria is **sensitive** for 1, 2 and **resist** 3
- Bacteria is _____ 4

2. Tube dilution methods

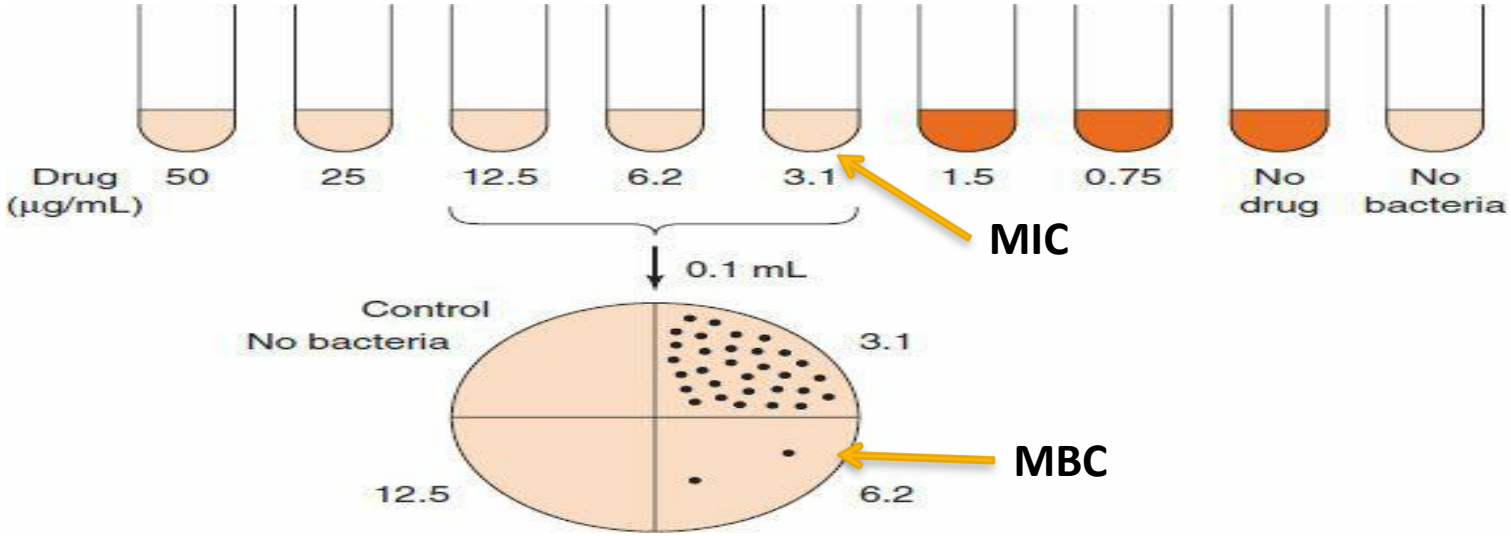
2. Tube dilution method

- This method includes inoculation of the organism isolated from the patient into a series of tubes containing two fold dilutions of the drug.
- It used to determine the minimal concentration (MIC) of antibiotic to inhibit or kill the MO.
- Minimal inhibitory concentration (MIC) is the lowest concentration of drug that prevents visible growth of the organism.
- Minimal bactericidal concentration (MBC) is identified as the smallest concentration of antibiotic that kills 99.9 of the MO.
- **MBC cannot be done without testing for MIC.**

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

Top: The patient's organism is added to tubes containing decreasing amounts of the antibiotic. After incubation at 37°C overnight, growth of the bacteria is observed visually. The lowest concentration of drug that inhibits growth (i.e., 3.1 µg/mL) is the MIC. However, at this point, it is not known whether the bacteria have been killed or whether the drug has only inhibited their growth.

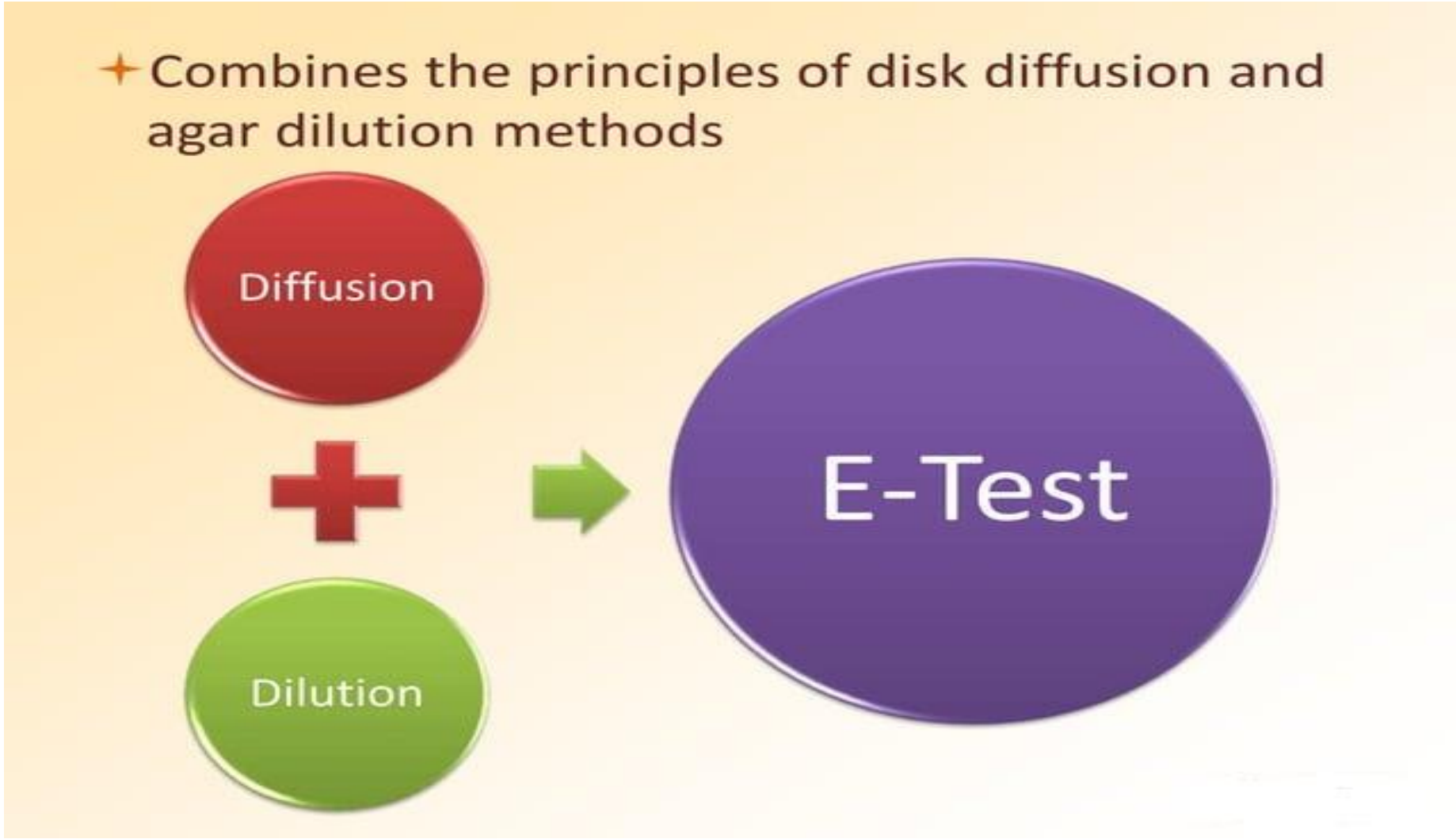
Bottom: To determine whether that concentration of drug is bactericidal (i.e., to determine its MBC), an aliquot (0.1 mL) from the tubes is plated on an agar plate that does not contain any drug. The concentration of drug that inhibits at least 99.9% of the bacterial colonies (i.e., 6.2 µg/mL) is the MBC.



3. Diffusion and Dilution Method

(The Epsilometer test)

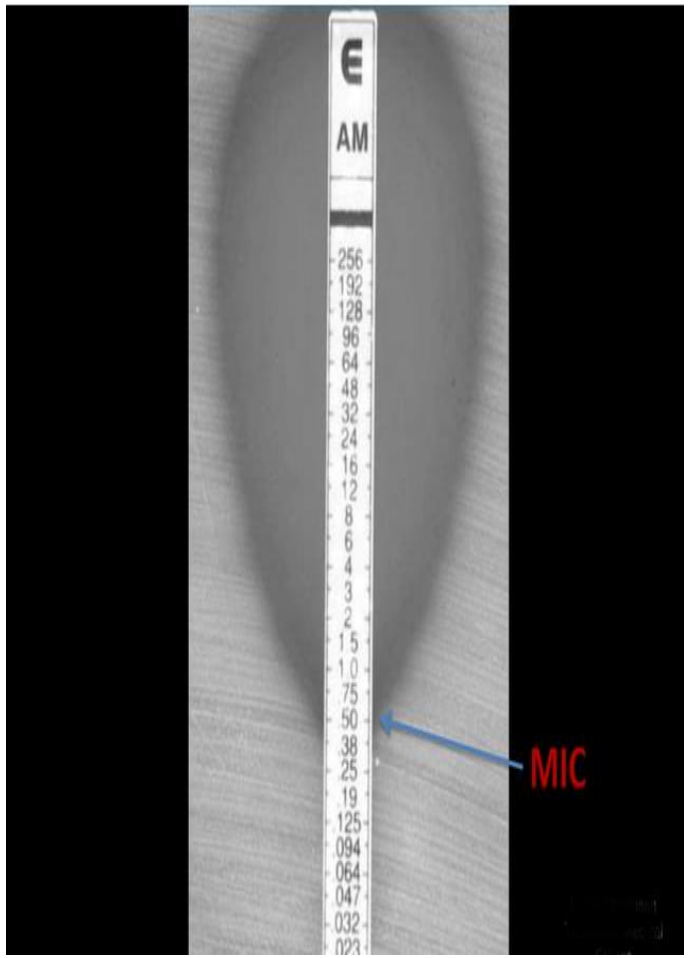
- Combines the principles of disk diffusion and agar dilution methods

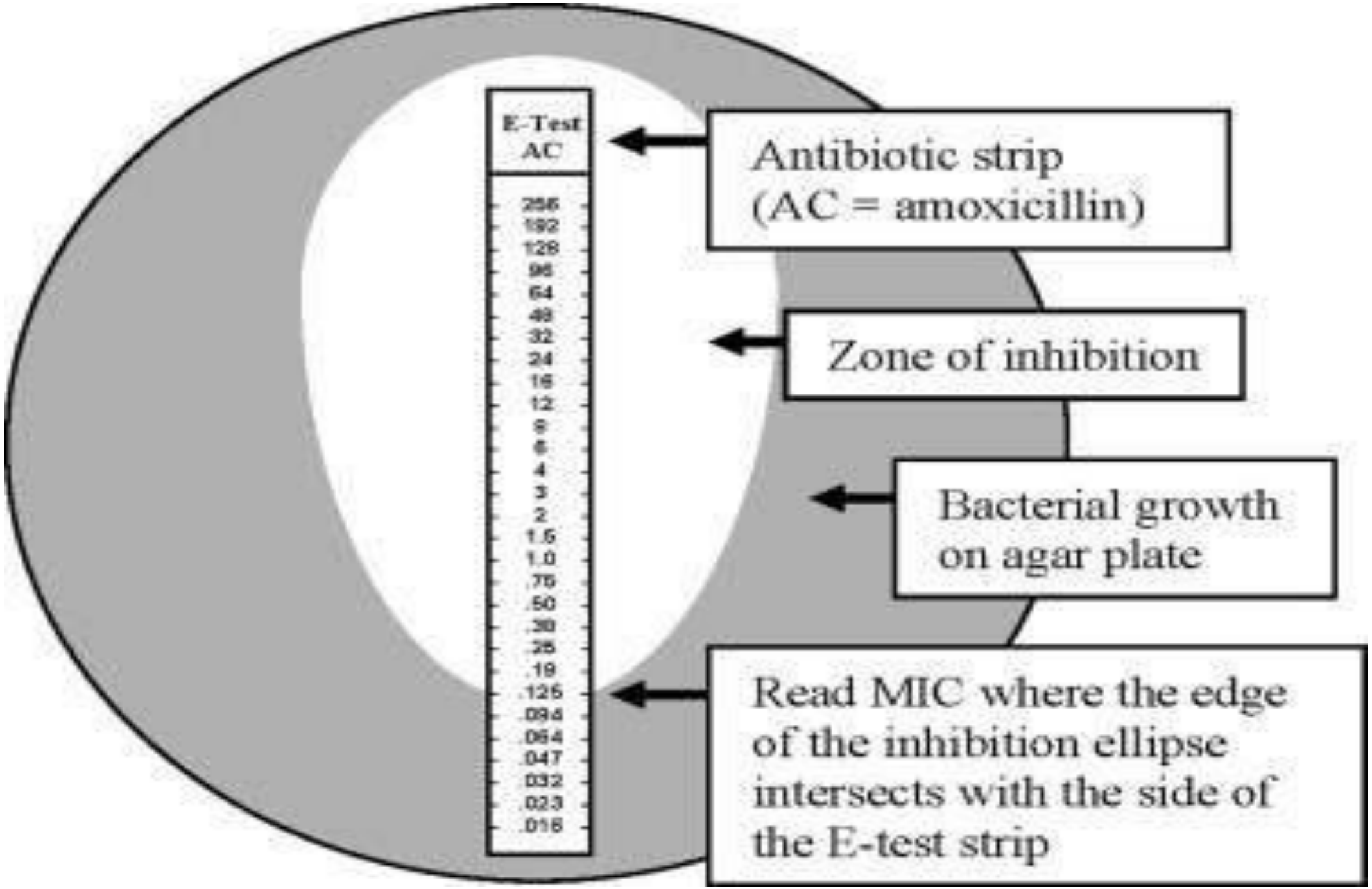


The Epsilometer test (Etest):

is a laboratory test used to determine whether or not a **bacterium** is susceptible to an antibiotic.

- The E test utilizes a rectangular strip that has been impregnated with a gradient concentration of drug to be studied.
- Bacteria cultured onto the Muller Hinton Agar (MHA) plate and the E test strip is laid on top; the drug diffuses out into the agar, producing an exponential gradient of the drug to be tested.
- After 24 hours of incubation, an elliptical zone of inhibition is produced and the point at which the ellipse meets the strip gives a reading for the (MIC) of the drug.




E-Test
AC
256
192
128
96
64
48
32
24
16
12
8
6
4
3
2
1.5
1.0
.75
.50
.38
.25
.19
.125
.094
.064
.047
.032
.023
.016
Antibiotic strip
(AC = amoxicillin)
Zone of inhibition
Bacterial growth
on agar plate
Read MIC where the edge
of the inhibition ellipse
intersects with the side of
the E-test strip


Antibiotic
Sensitivity Testing
Choose the right drug!
Get faster cure!
Prevent drug resistance!

Thank you