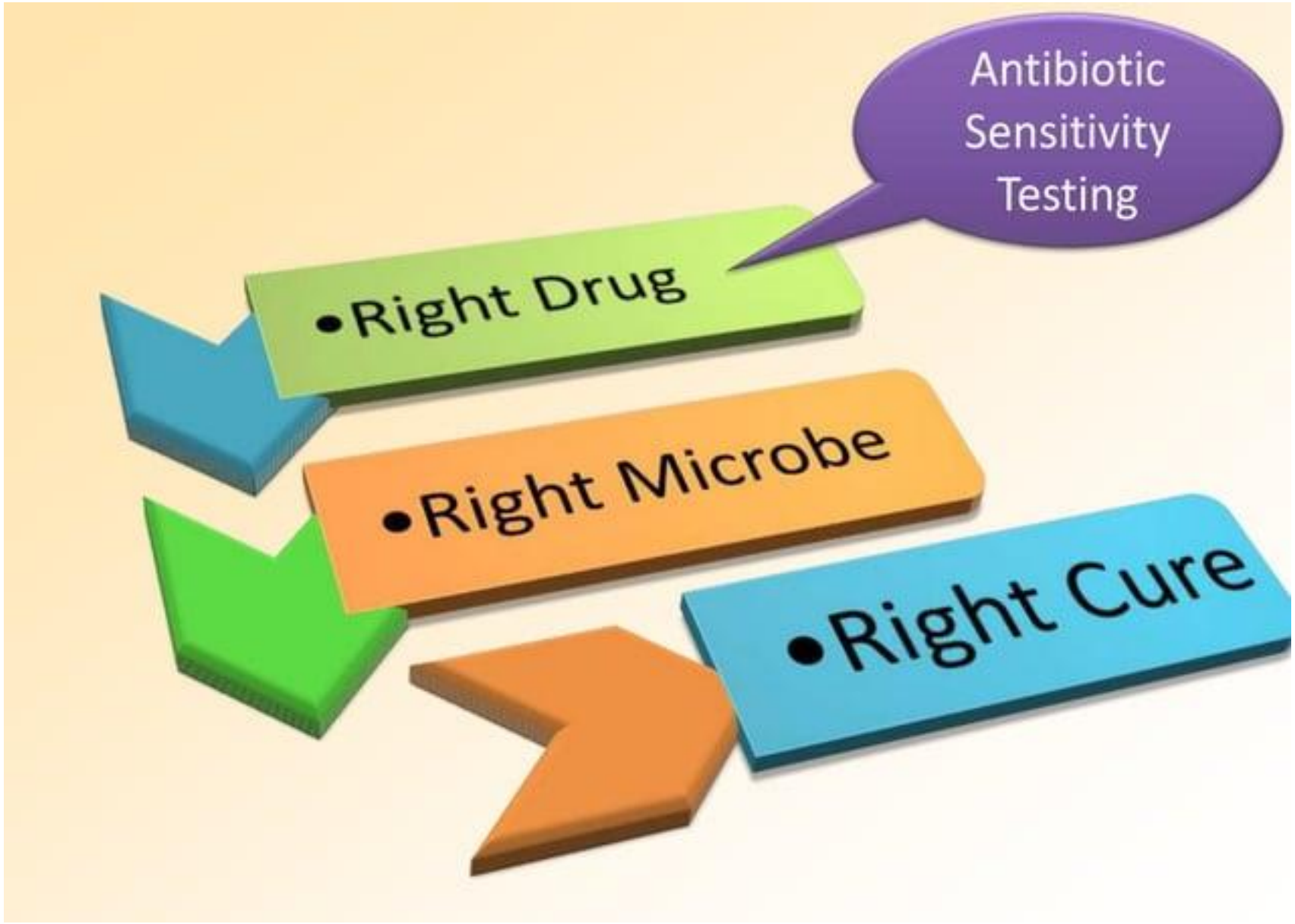
Lab – 7

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

Diffusion methods

Diffusion methods

Kirby-Bauer method or disk diffusion antibiotic sensitivity testing

- 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.

Agar disk diffusion method 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.

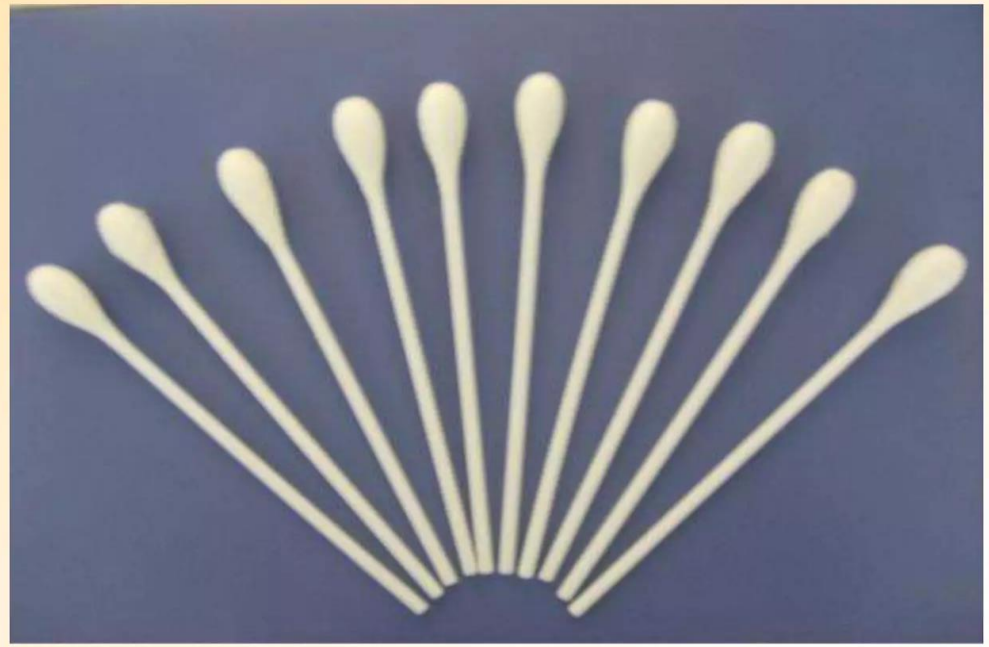
4. Flame sterilize the forceps with alcohol before picking up the discs.

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.

Cotton Swabs



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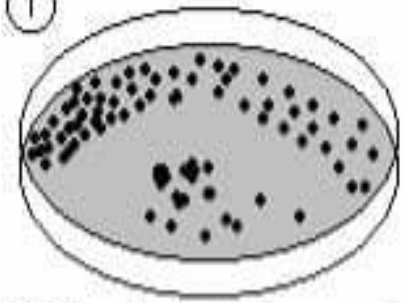
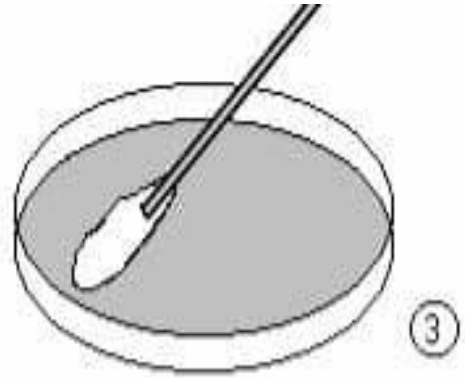
Procedure for disk diffusion testing



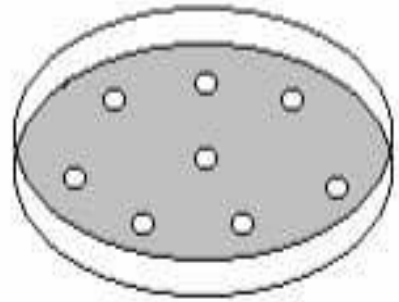
2-3 identical colonies are picked from the plate and transferred to the broth

The tube is incubated for the bacteria to grow.

The inoculum density is standardized using McFarland standard

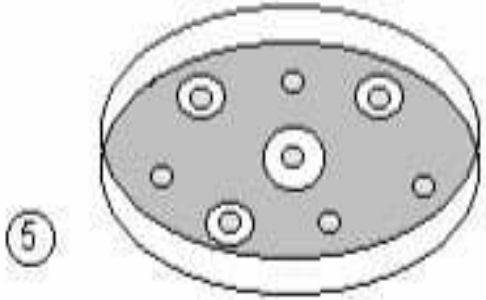


A cotton swab dipped in the inoculum suspension is swabbed over the entire surface of agar to give a lawn culture.



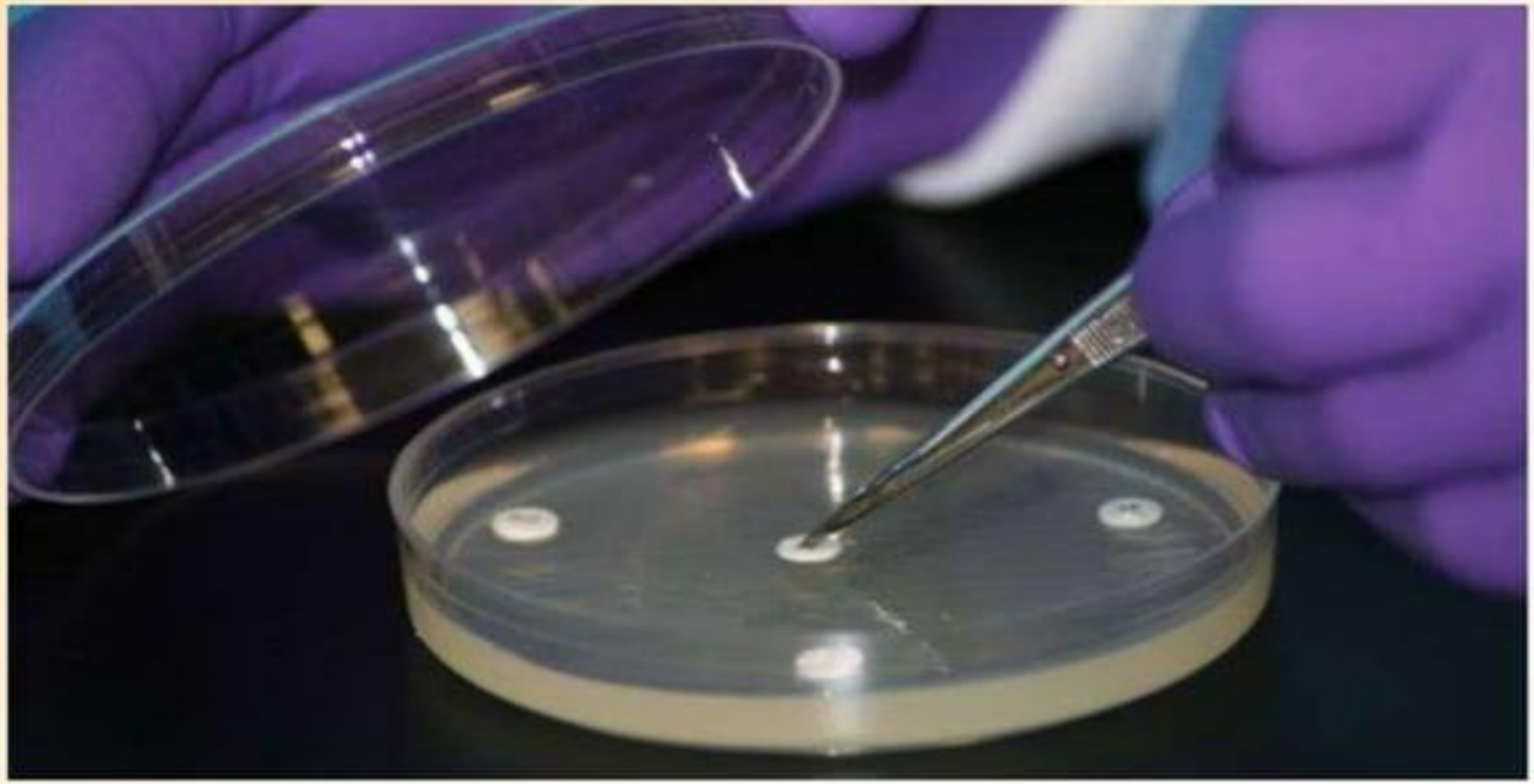
Filter paper disks containing known antibiotic in known concentration is placed

Plate incubated at 37C overnight



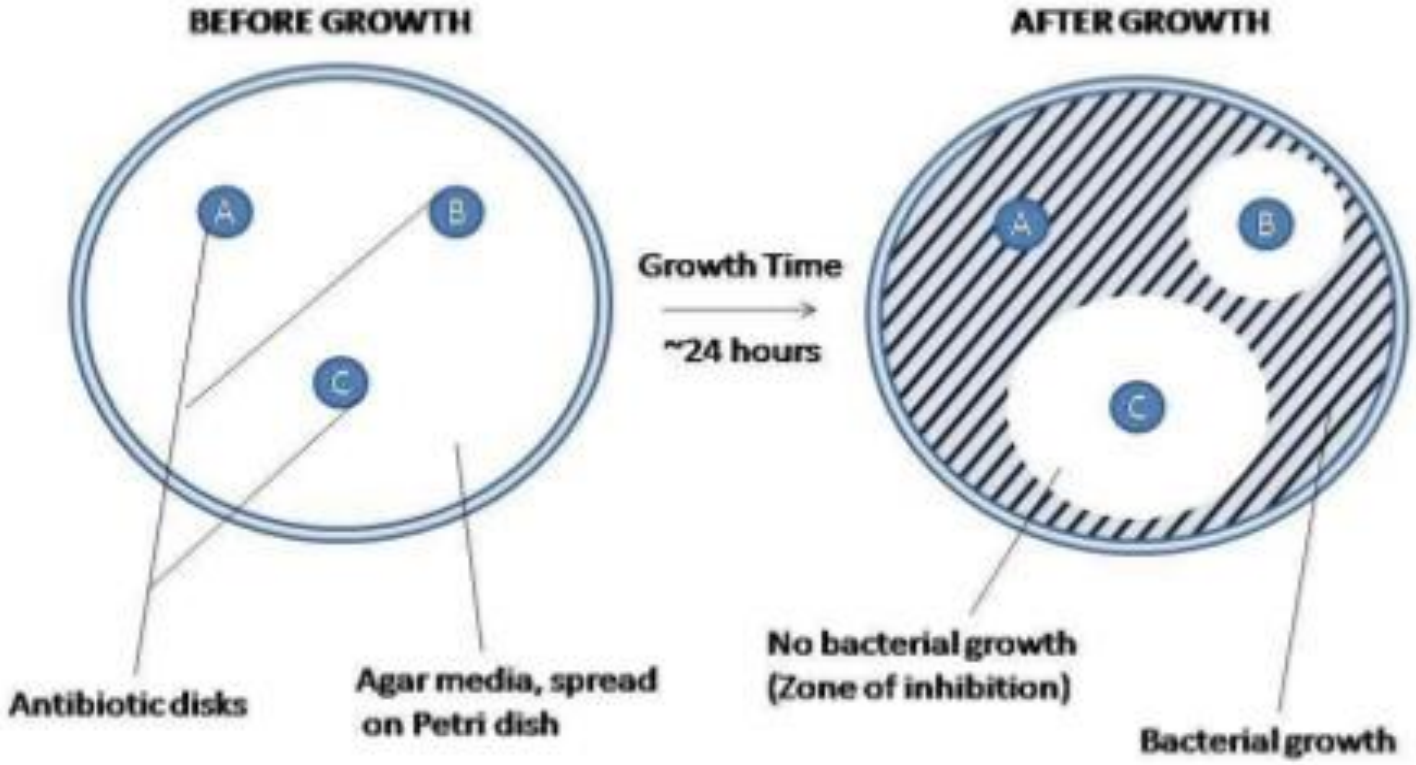
Zone diameter around the disk are measured and result read from Kirby Bauer chart

Antibiotic Disks

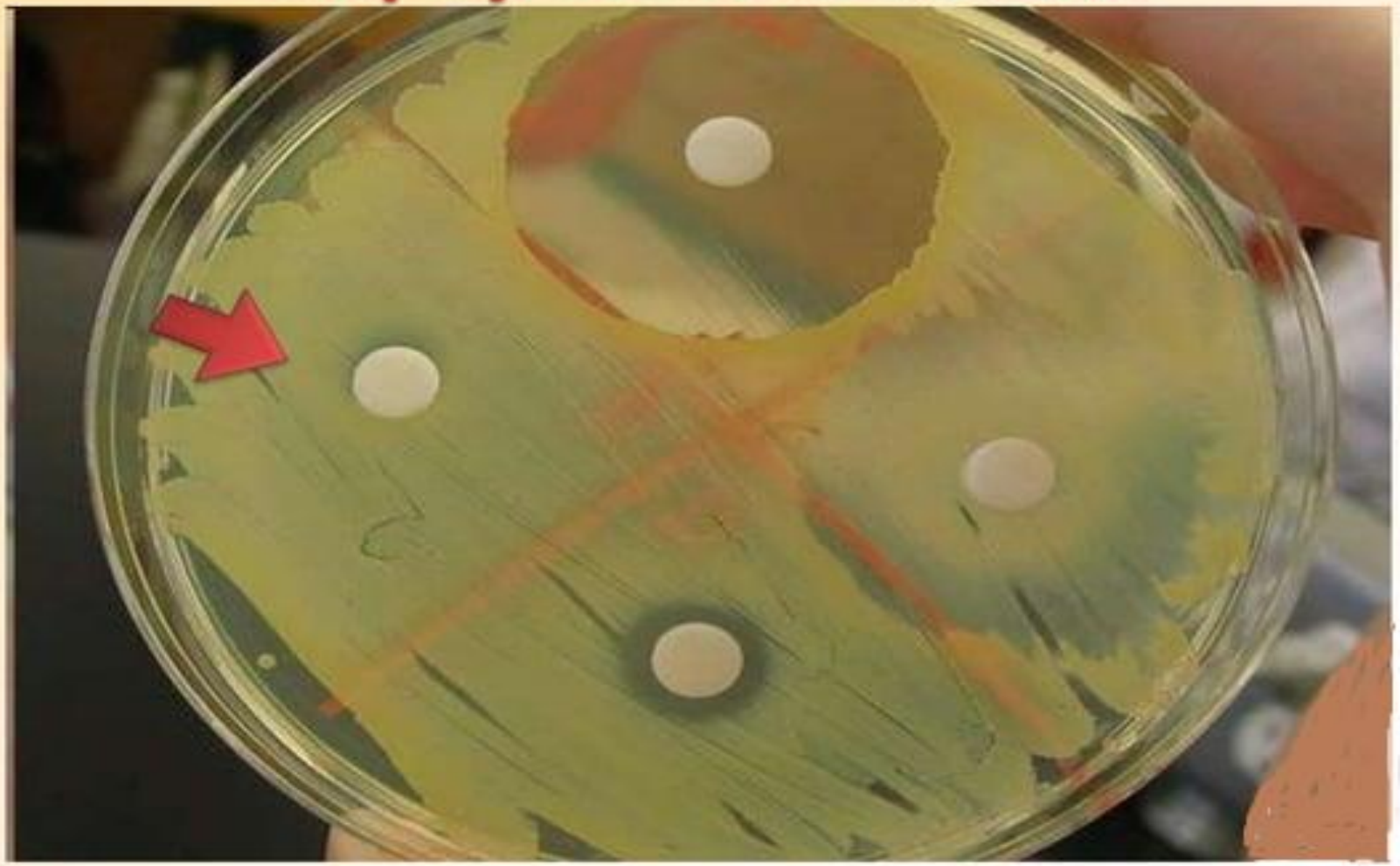


Result

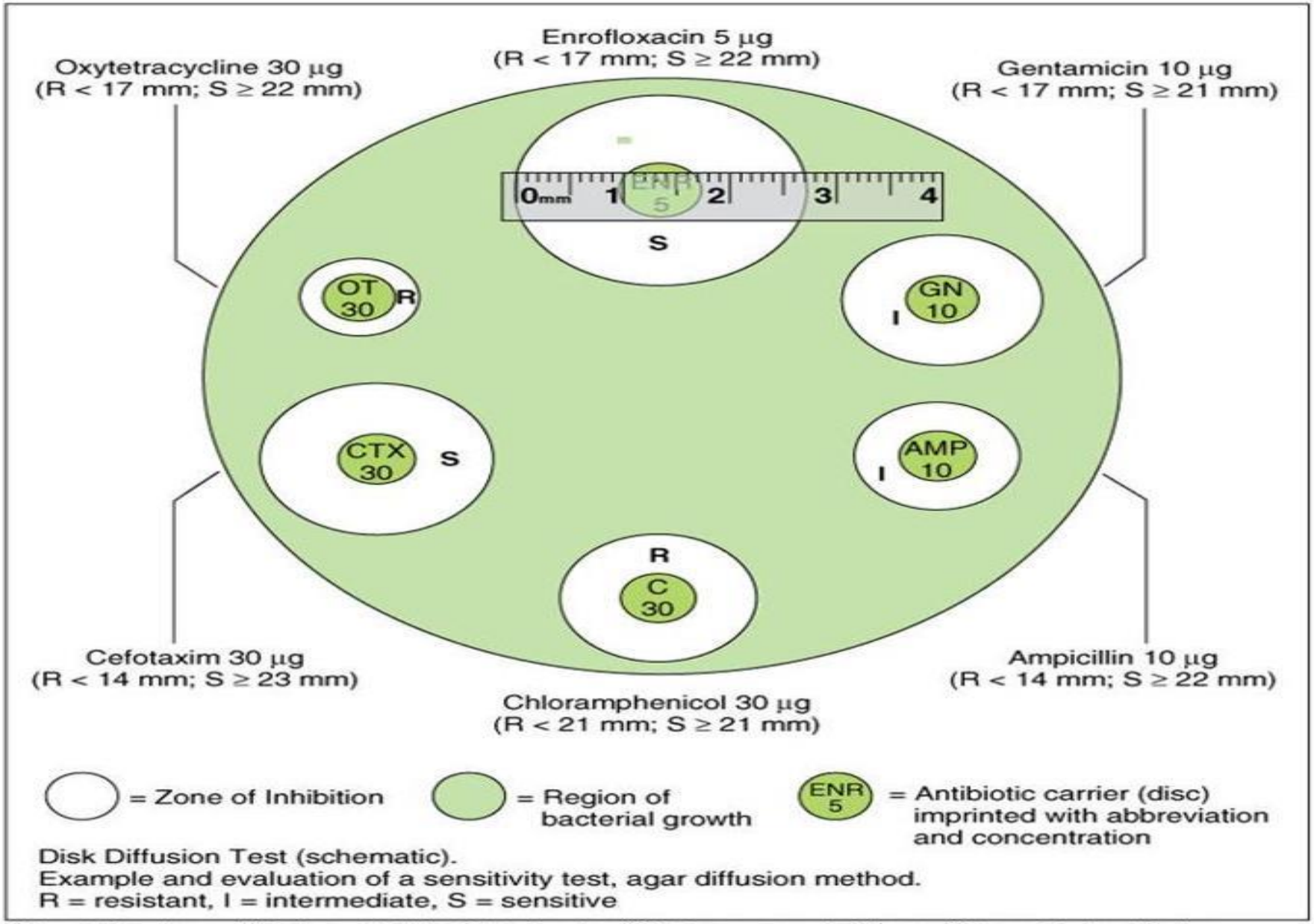

Antibiotic


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

Methicillin resistance in Staphylococcus aureus



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

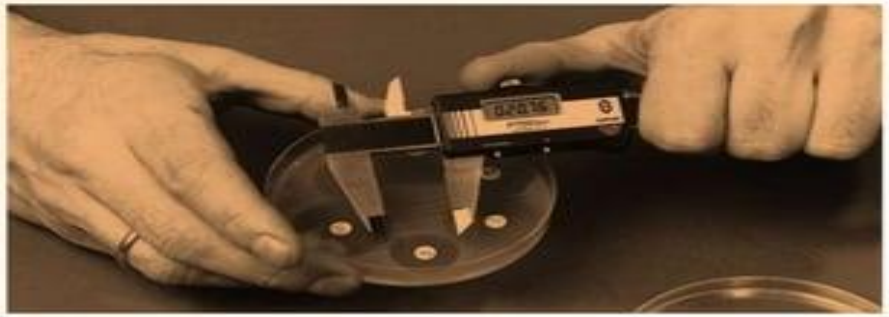
(a)

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

Measurement of diameter

Using a ruler

- The **diameter of the zone of inhibition** is measured using a ruler or a pair of calipers.
  - This diameter is interpreted according to the critical diameters.

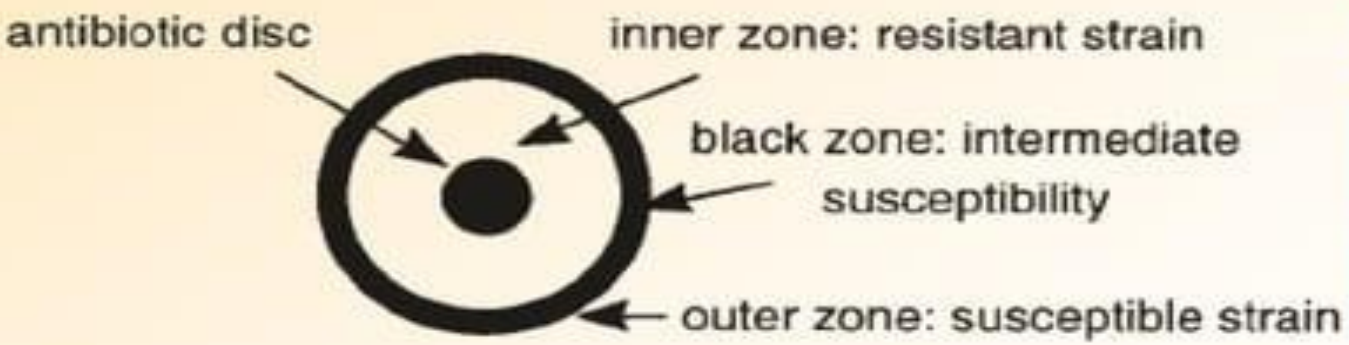


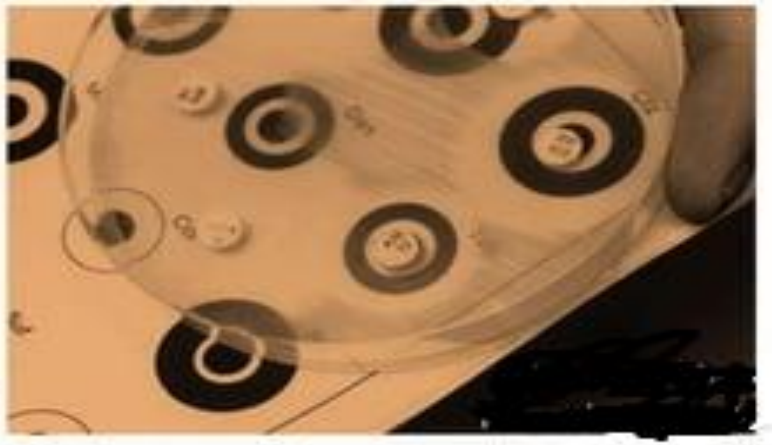
Using a ruler measure the diameter of any zones of inhibition and record your results, the results must be compared with values listed in standard charts as shown in the interpretative chart below:

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

Using a template

- Result interpretation
  - **Susceptible**
    - When the edge of the zone of inhibition is **OUTSIDE** the black circle.
  - **Resistant**
    - When there is no zone, or when it lies **WITHIN** the white circle.
  - **Intermediate**
    - When the edge of the zone of inhibition lies **ON** the black circle.

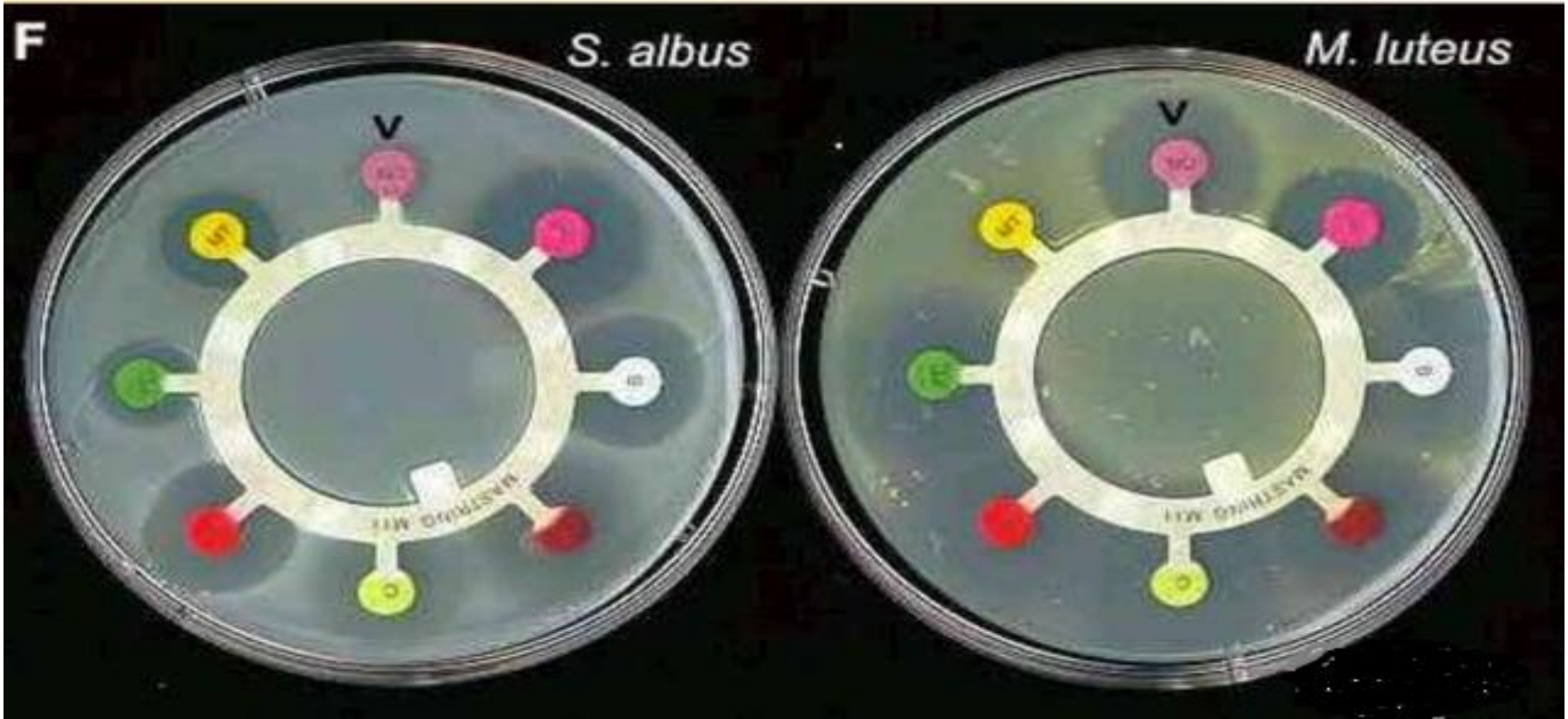




- Antibiotics may be also placed in wells made in the agar medium by a cork borer.

- Or antibiotics may be incorporated with the melted agar and poured together in Petri dishes, in this case each dish will contain only on antibiotic.

Strips of multiple antibiotics can be tested in one go



Dilution methods

Dilution methods

- Used to determine the **minimal concentration** of antibiotic to inhibit or kill the microorganism.
- Achieved by dilution of antibiotic in either agar or broth media.

Minimum inhibitory concentration

- The lowest concentration of drug that ***inhibits the growth*** of the bacteria isolated from the patient.
- The MIC is determined by inoculating the organism isolated from the patient into a series of tubes or cups containing progressive dilutions of the drug.

MIC: Minimum inhibitory concentration

The minimal bactericidal concentration (MBC) of an antibacterial which is defined as the maximum dilution of the product that will kill a test organism can be determined by subculturing last clear MIC tube onto growth medium that does not contain antibiotic, and examining for bacterial growth.

The MBC is identified as the smallest concentration of antibiotic that prevents any growth of the test bacterium (i.e., kills).

MBC cannot be done without testing for MIC.

Tube dilution

Patient's organism is added to tubes containing decreasing amounts of the antibiotic

Incubation
At 37°C overnight

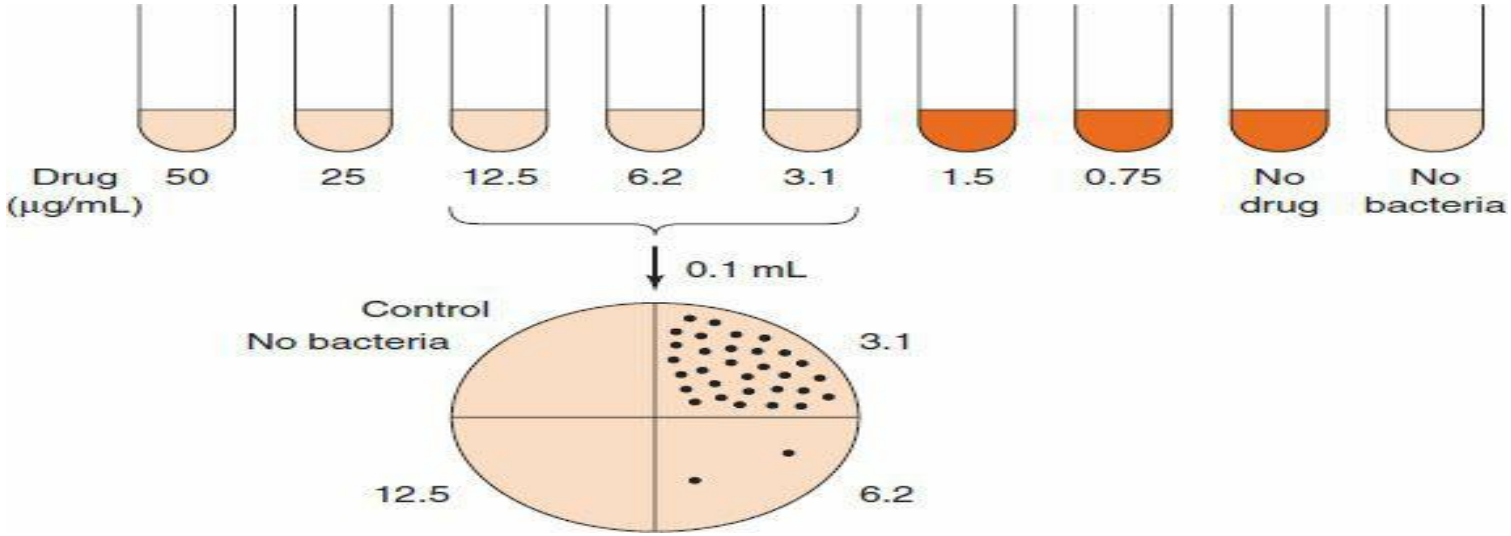
Lowest concentration of drug that inhibits growth is the MIC

Dilution method

The Dilution method is used to determine the minimal inhibitory concentration (MIC) of an antimicrobial to inhibit or kill the bacteria/fungi and is the reference for antimicrobial susceptibility testing

The MIC is determined by inoculating the organism isolated from the patient into a series of tubes containing twofold dilutions of the drug.

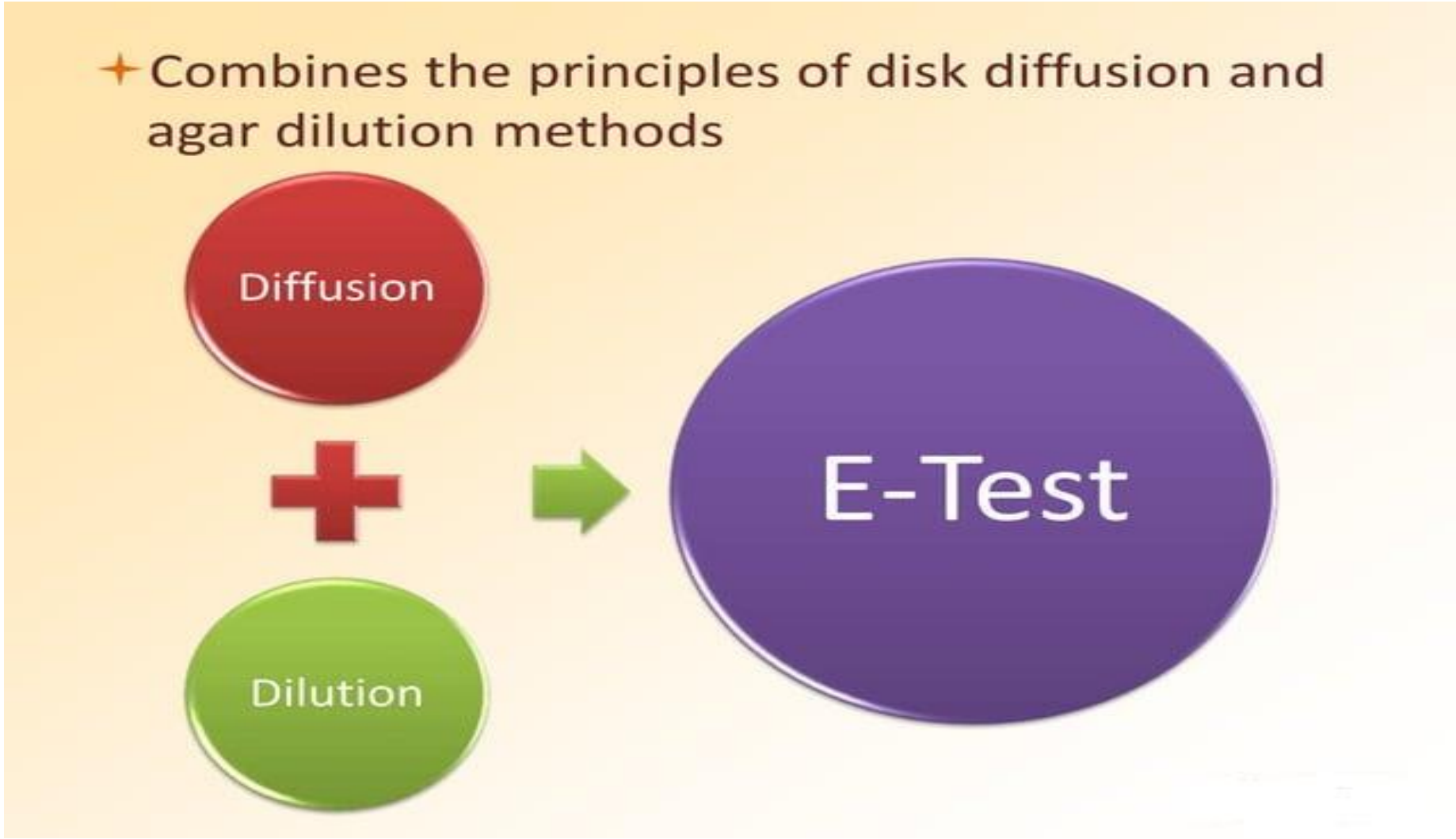
After incubation, the **lowest concentration of drug that prevents visible growth** of the organism is the MIC.



Diffusion and Dilution Method

(The Epsilometer test)

- Combines the principles of disk diffusion and agar dilution methods



Other methods to test antimicrobial susceptibility include the **E-test** also based on antibiotic diffusion.

The Epsilometer test (usually abbreviated 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 the drug to be studied.
- A lawn of bacteria is inoculated onto the surface an agar 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.
- There is an exponential scale printed on the strip.
- 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 Procedure

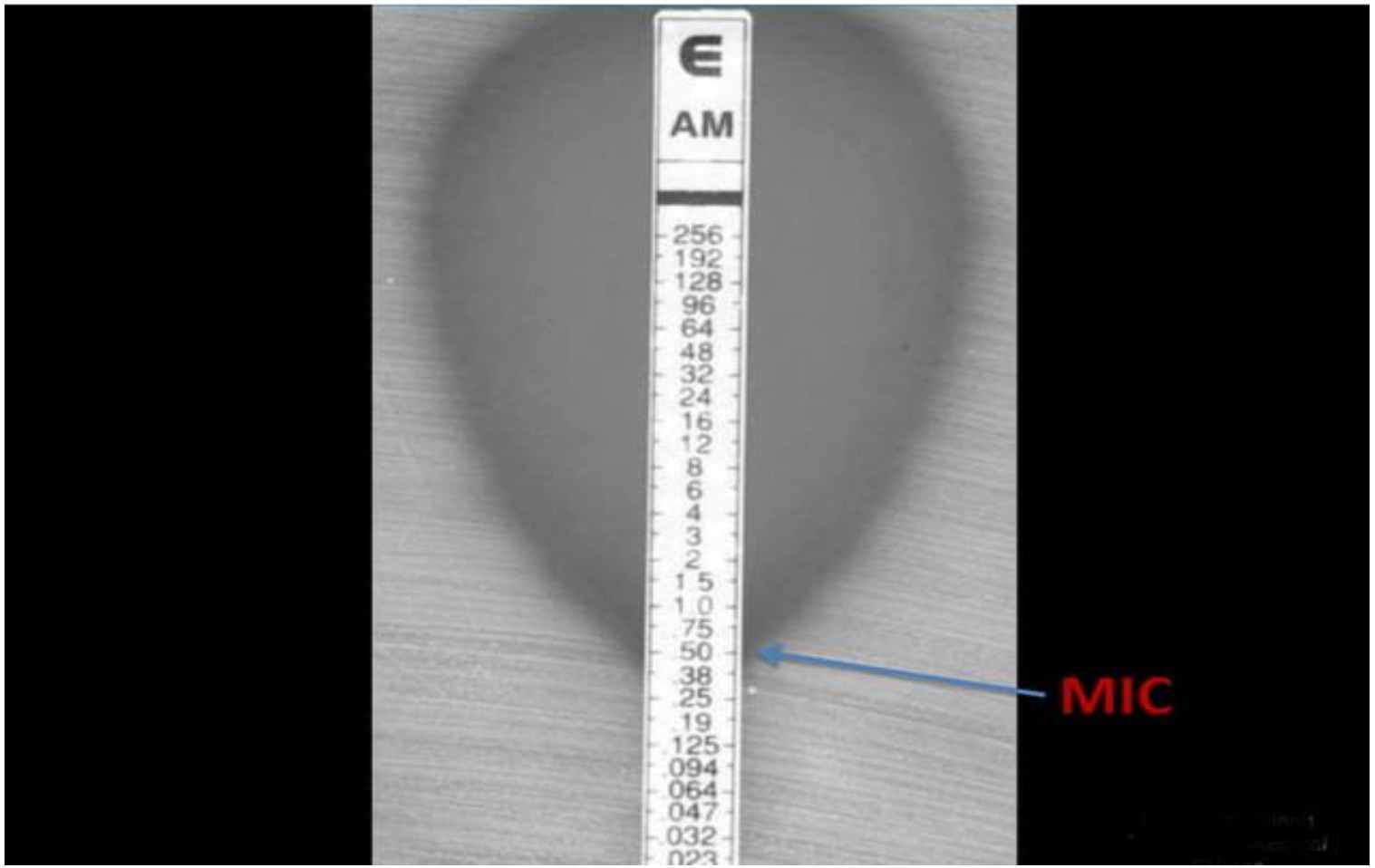
Apply E-Test strip on an inoculated agar plate

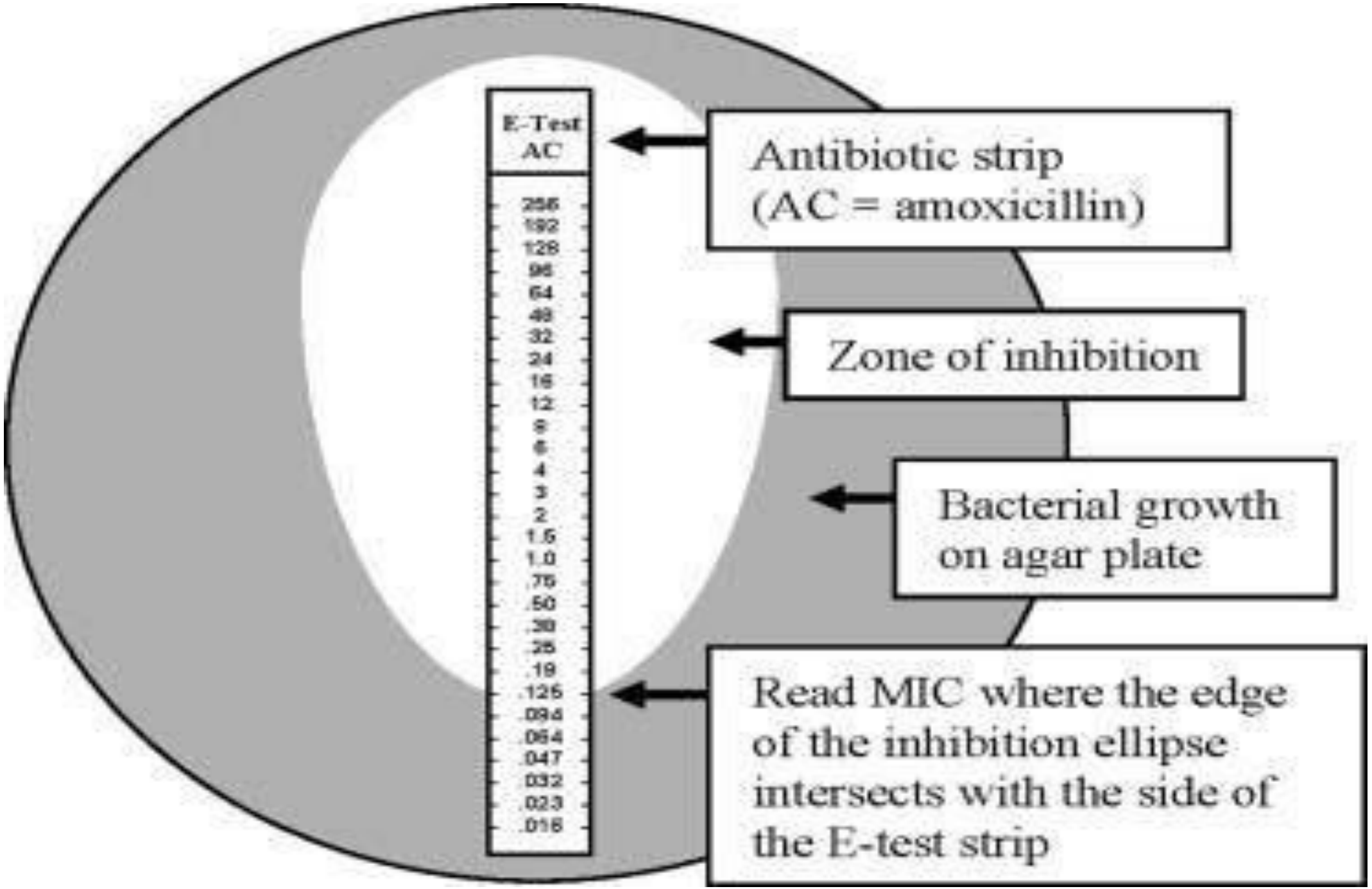
Immediate release of drug

Incubation of plate

Symmetrical inhibition ellipse is produced

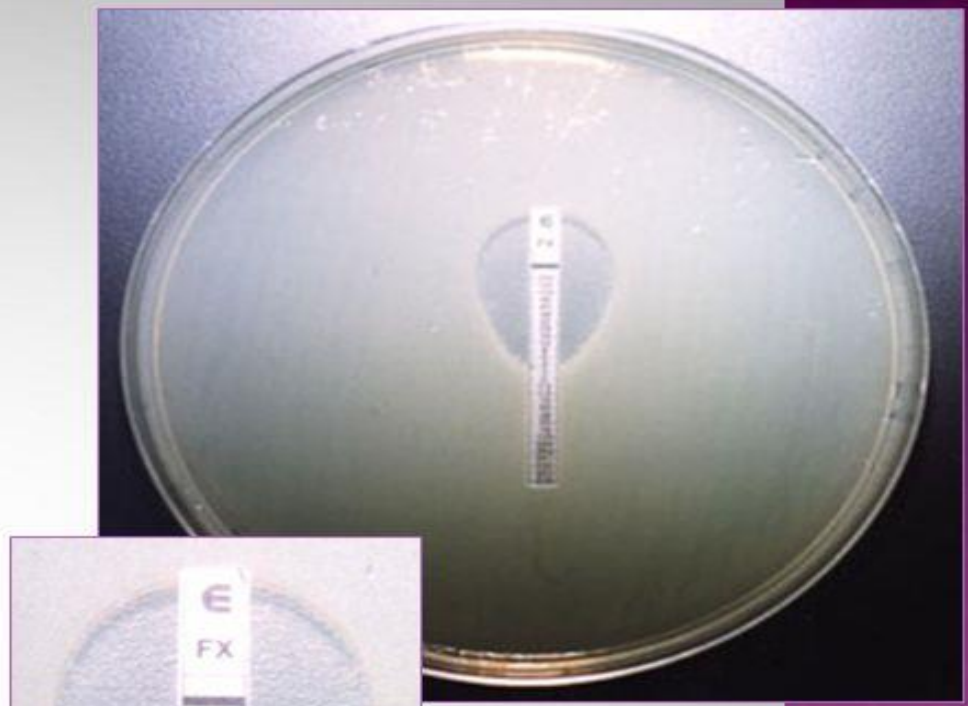
The intersection of the inhibitory zone edge and the calibrated carrier strip indicates the MIC with inherent precision and accuracy.


E
AM
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
MIC


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

EPSILOMETER TEST
ETEST (STRIP TEST)

a rectangular strip that has been impregnated with antibiotic, **used to determine MIC.**




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

Thank you