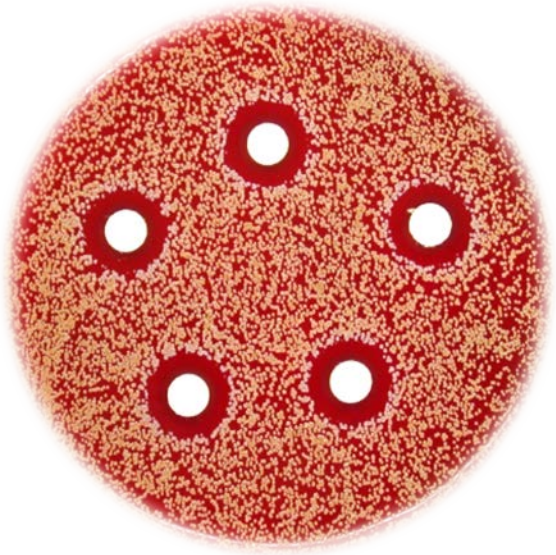
Lab 5.



Assessing Antibiotic Effectiveness

MICROBIAL DIAGNOSIS

320 MIC

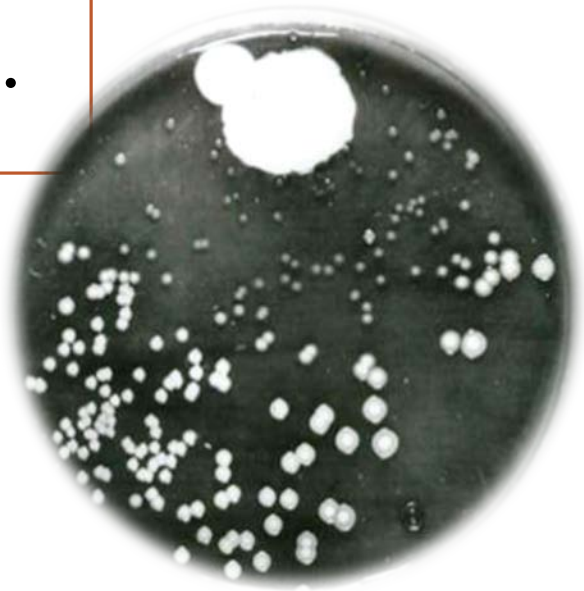
PRACTICAL

Antibiotic have become a standard method used by physician to treat bacterial disease.

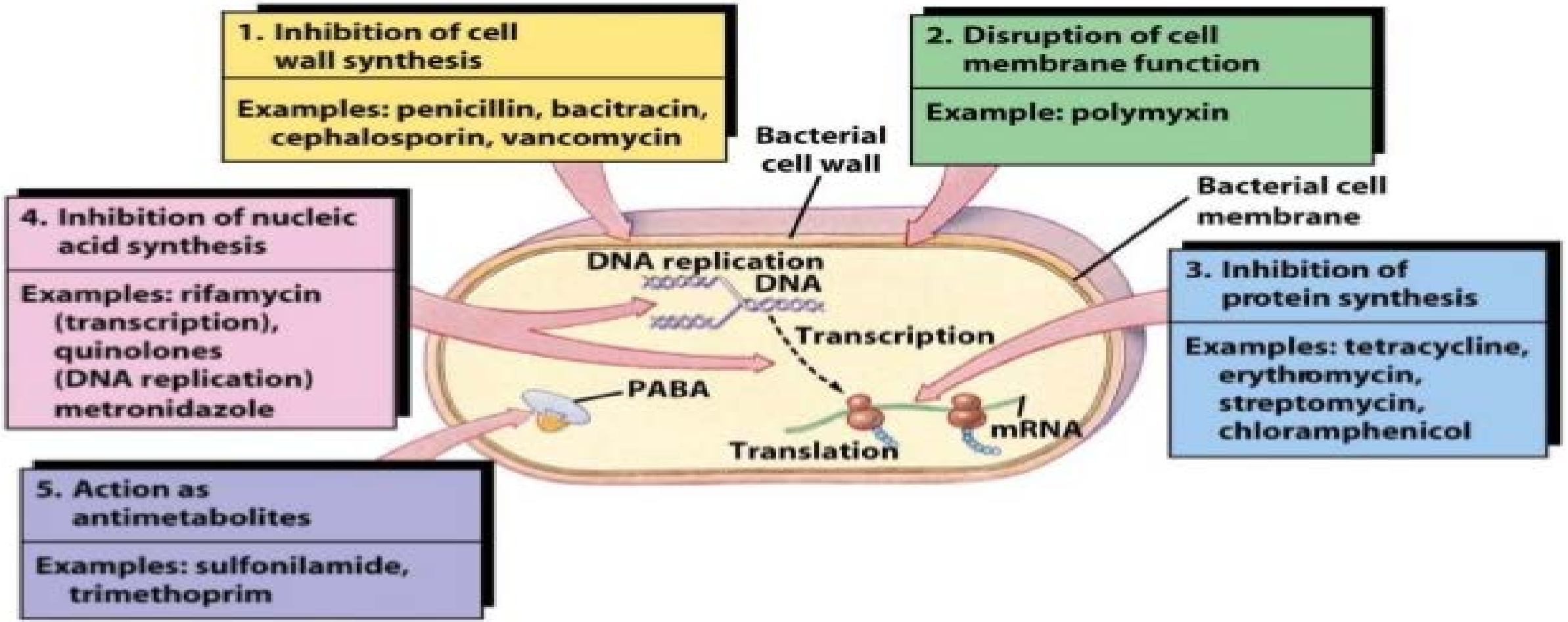
The first antibiotic was founded by **Alexander fleming**. It was penicillin that produced by his molds over 60 years ago.

Since the discovery of penicillin, many other useful antibiotics have been developed.

Each antibiotics has a **specific mechanism** of action against bacteria, the action may differ among bacteria.



Antibiotic Mechanisms of Action



Depending on the range of bacterial species susceptible to these agents, Antibiotics are classified to :

1. **Broad spectrum antibiotics**
2. **Narrow spectrum antibiotics**

Classification according to spectrum of activity

Broad spectrum	Narrow spectrum
• An active against both Gram positive and Gram negative organisms. • **For example :** Tetracyclines	• Have limited activity and are primarily only useful against particular species of microorganisms. • **For example :** ➢ Polymixins → Gram negative ➢ Bacitracin → Gram positive

1. **Dilution methods**
2. **Disk diffusion method**
3. **E-test**

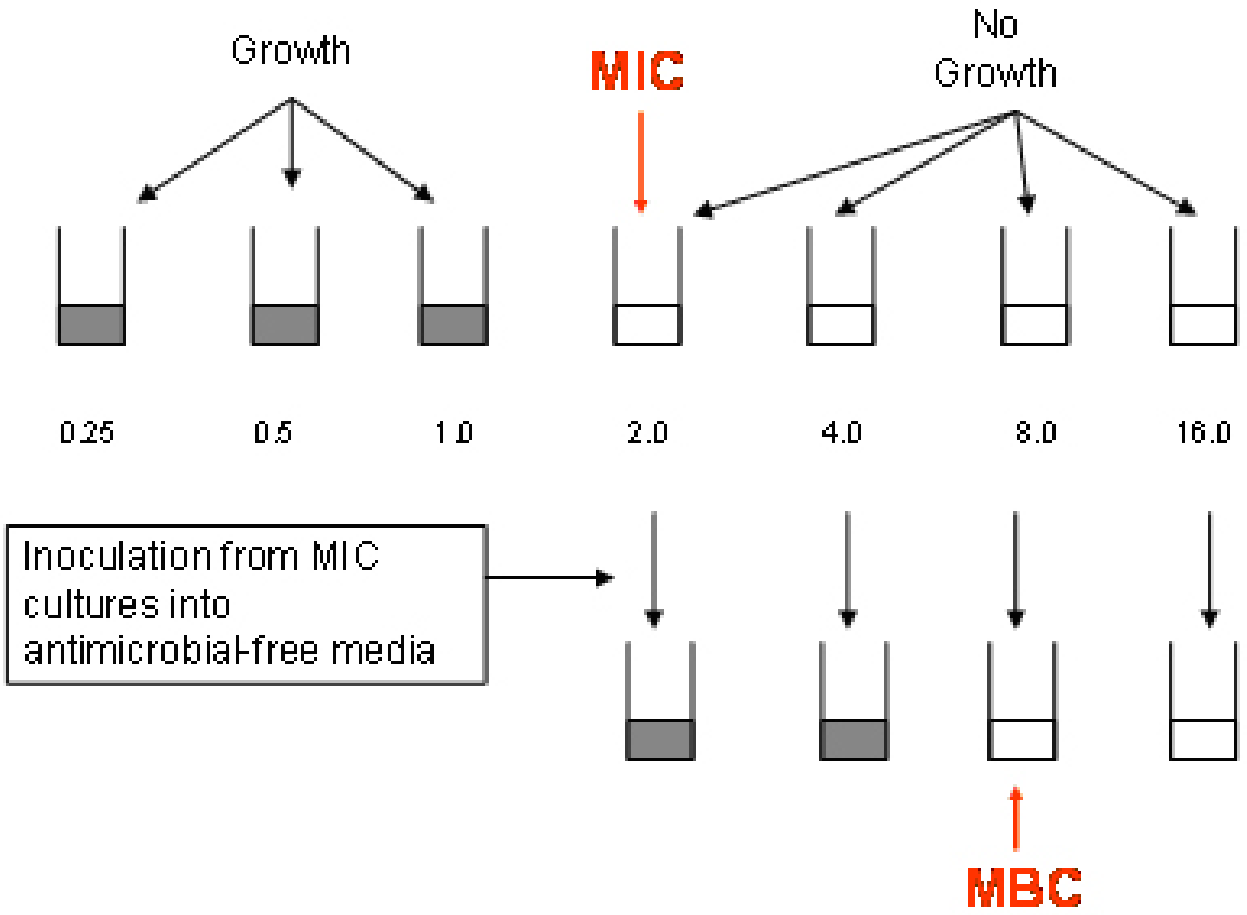
1. Dilution Method :

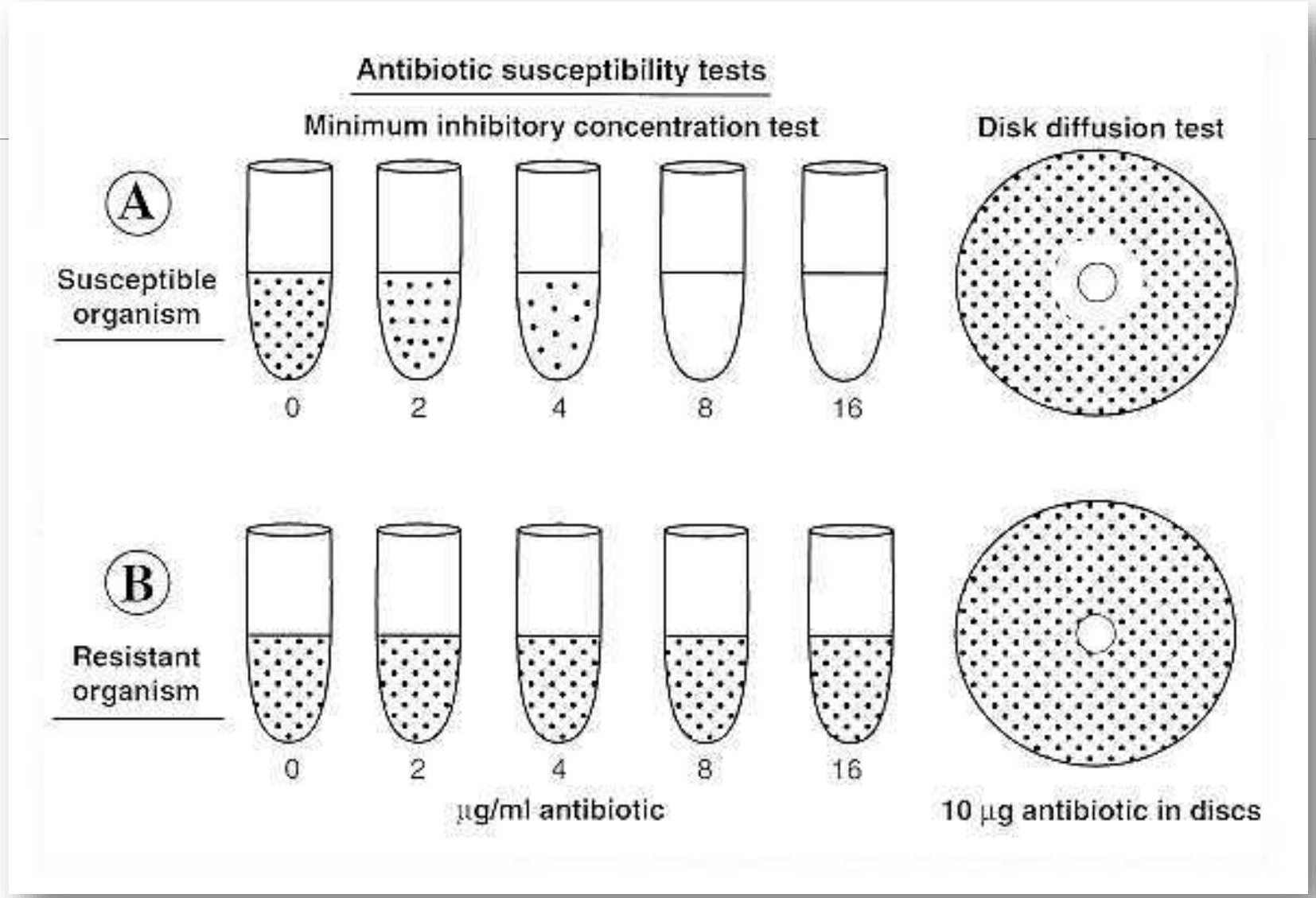
The Broth dilution method involves subjecting the isolate to a series of concentrations of antimicrobial agents in a broth environment.

The lowest concentration at which the isolate is completely inhibited is recorded as the minimal inhibitory concentration **MIC**.

The MIC is thus the minimum concentration of the antibiotic that will inhibit this particular isolate.

Serial Dilution Susceptibility Testing




Antibiotic susceptibility tests
Minimum inhibitory concentration test
Disk diffusion test
A
Susceptible organism
0
2
4
8
16
B
Resistant organism
0
2
4
8
16
µg/ml antibiotic
10 µg antibiotic in discs

2. Disk Diffusion Method (Kirby Bauer Test):

K-B Test is routinely done to monitor the prevalence of antibiotic resistant bacteria.

Observe for a trend in order to take precautionary measures.

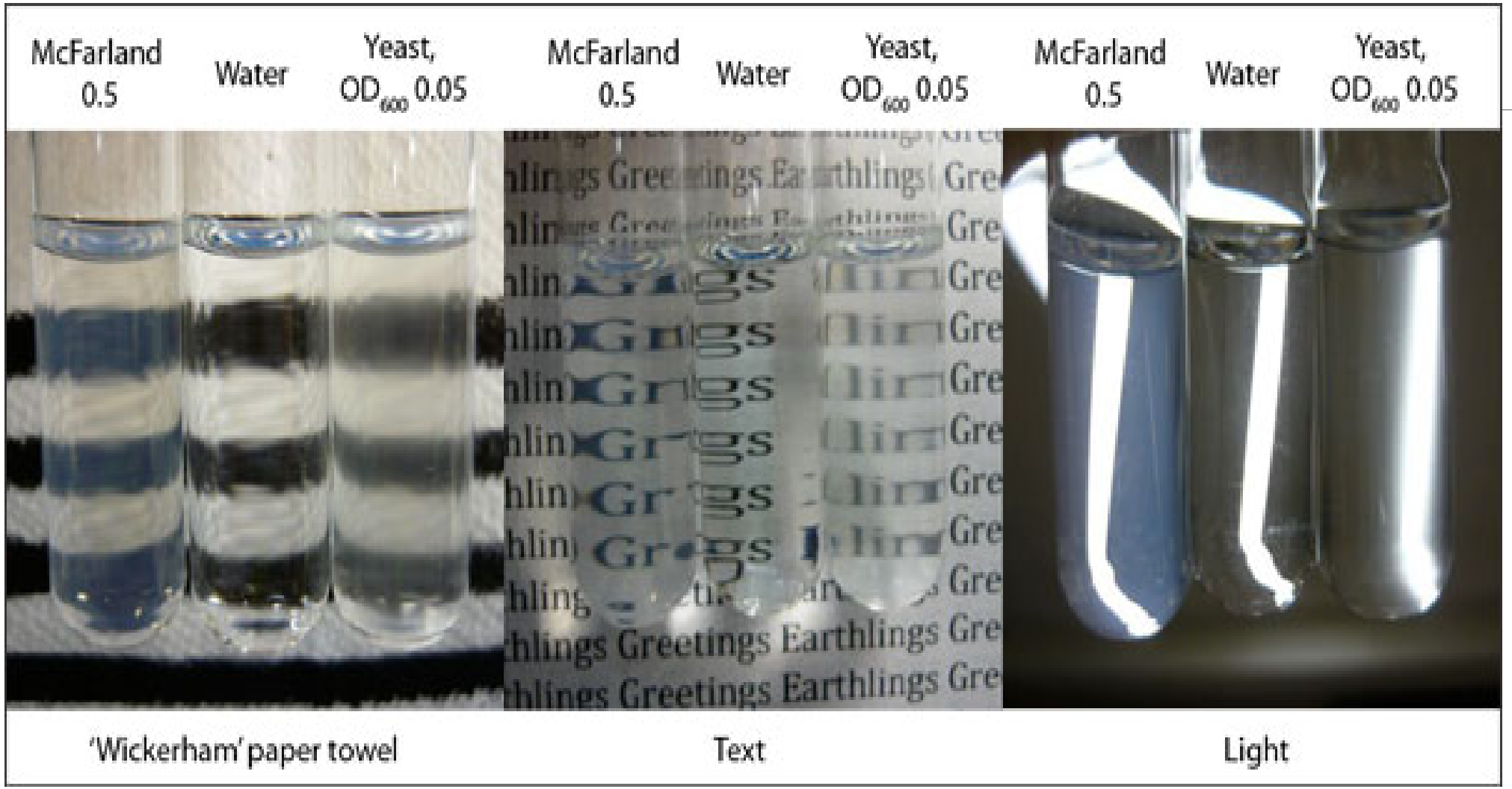
For example :

- development of new drugs
- determining the molecular basis for resistance and modify existing drugs accordingly

❑ Procedures :

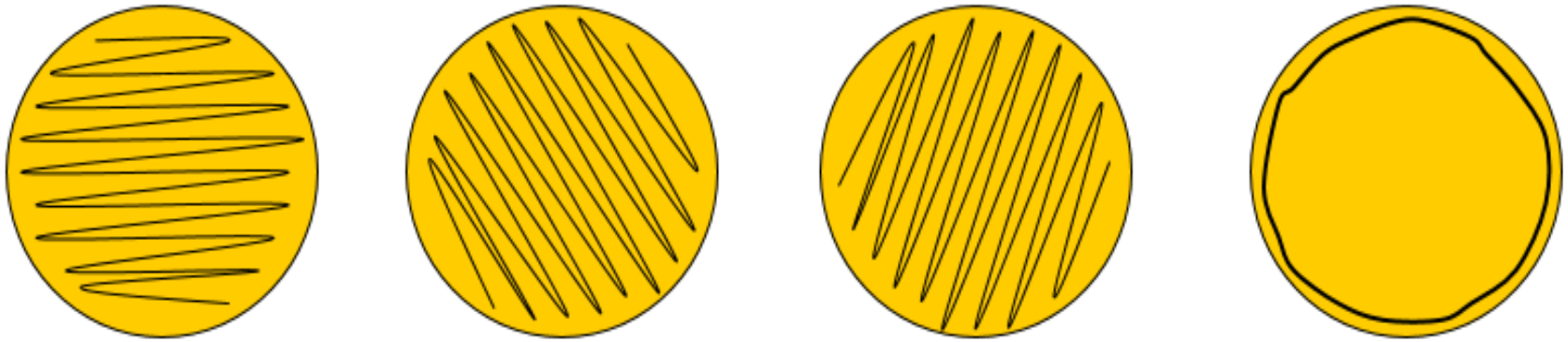
Prepare a pure culture (18-24 hrs.) of the sample on a non-selective medium

Adjust **turbidity** until it is equivalent to the **0.5 McFarland** Turbidity Standard.


McFarland 0.5
Water
Yeast, OD_{600} 0.05
McFarland 0.5
Water
Yeast, OD_{600} 0.05
McFarland 0.5
Water
Yeast, OD_{600} 0.05
'Wickerham' paper towel
Text
Light

Within 15 minutes of adjusting the turbidity, dip a sterile cotton swab into the sample.

Streak a lawn of bacteria on Mueller-Hinton agar

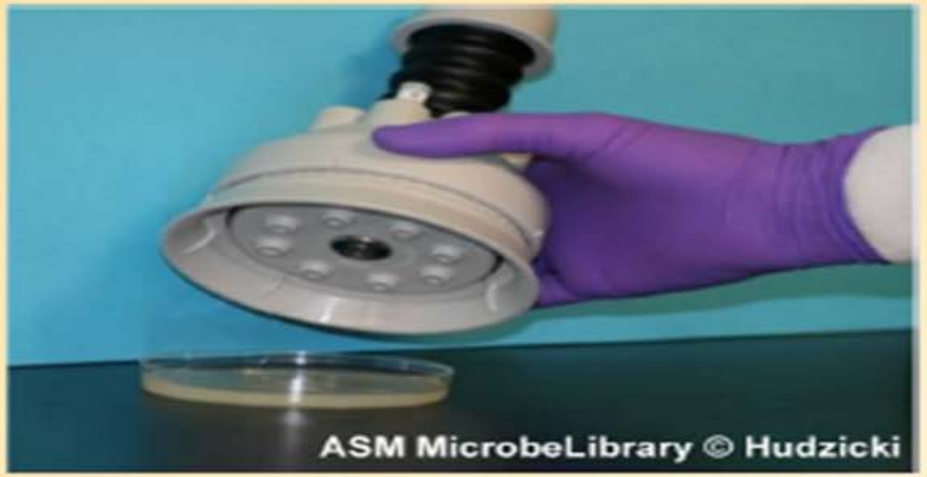


Leave the lid agar for 3-5 minutes (no more than 15 minutes) to allow plate to dry.

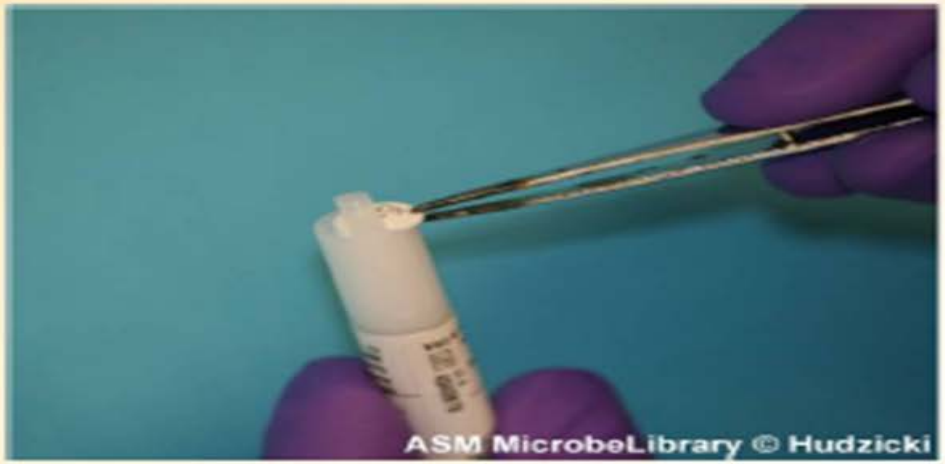
Apply antibiotic impregnated disks on the bacterial lawn.

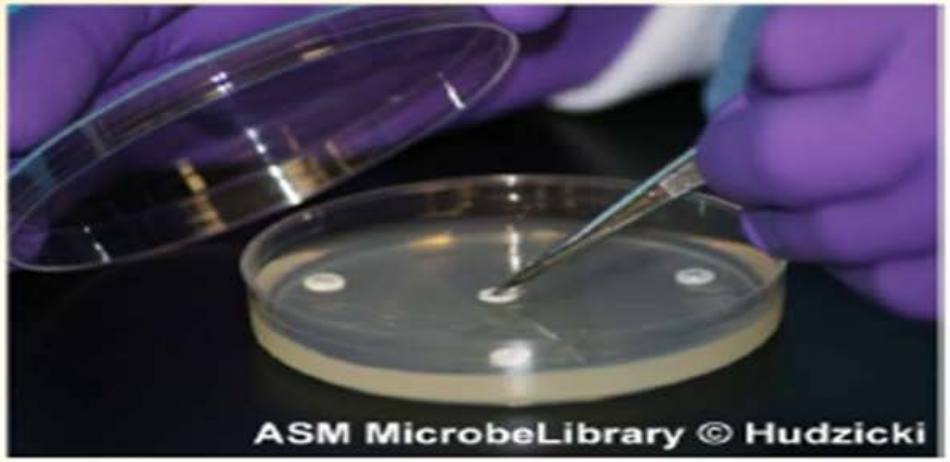
Important: where the disk drops is where it stays.

Incubate for 16 – 18 hours at 37°C unless otherwise instructed.



placement of antibiotic disks using an automated disk dispenser





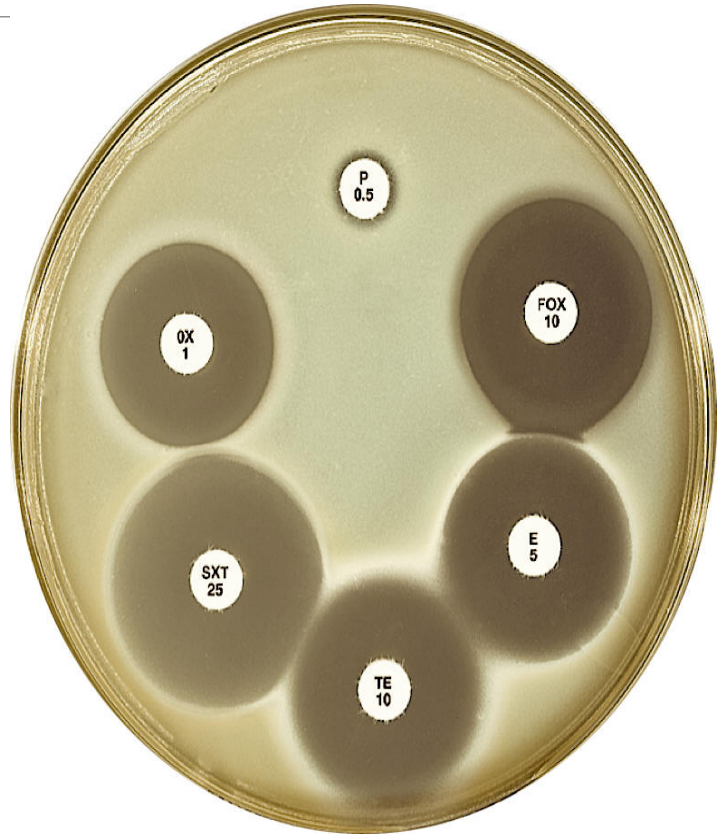
placement of antibiotic disks using forceps

❑ Result :

Antibiotics diffuse out onto the agar.

Concentration of antibiotics decrease as they diffuse further away from the disks

After incubation, observe for a clearing on the bacterial lawn (zone of inhibition)



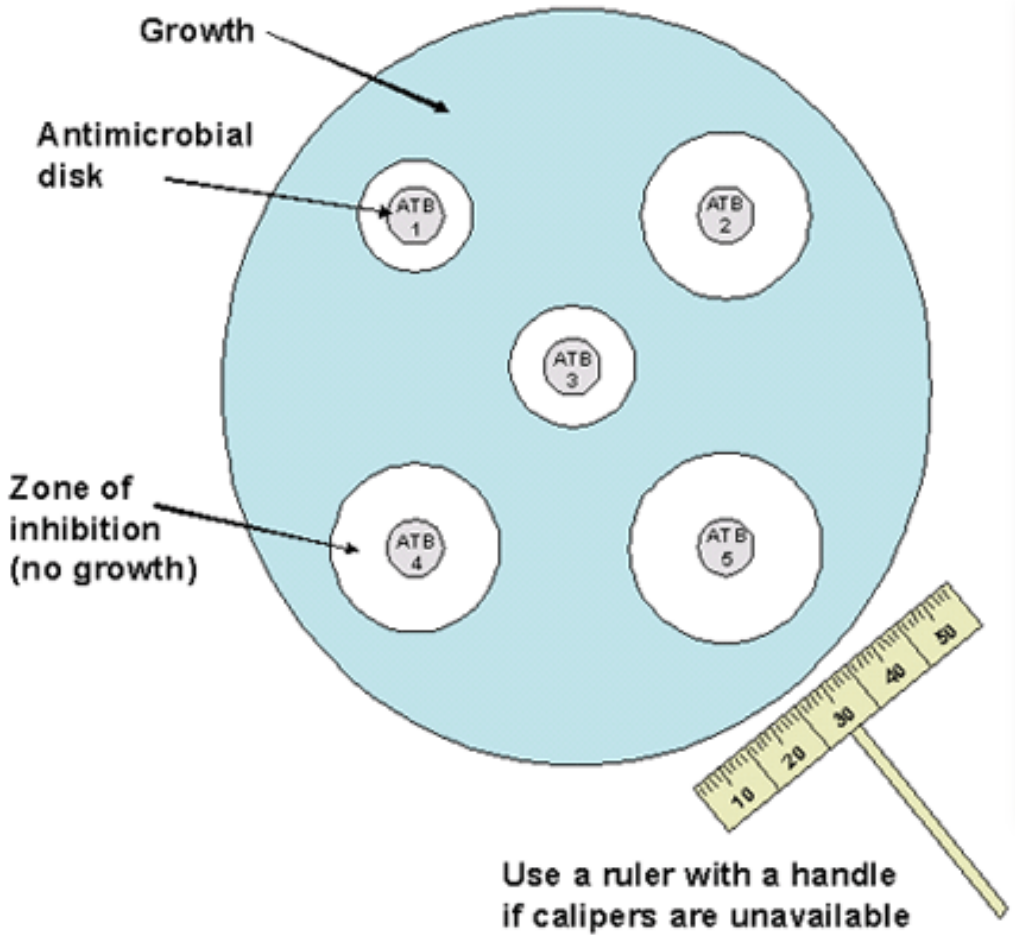
❑ Result :

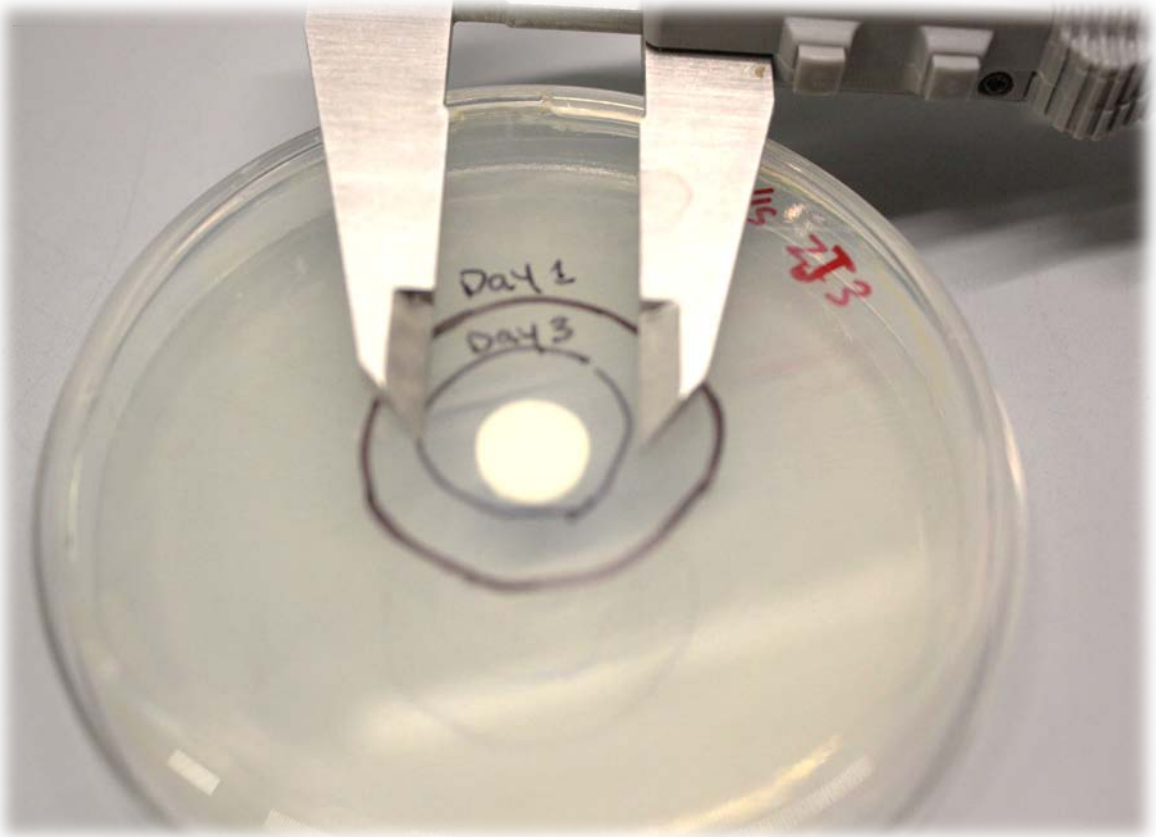
Measure the diameters of the zone of inhibition

Interpret the results as “resistant” or “susceptible” according to the guideline provided by the NCCLS

Interpretation of the zone of inhibition is different for each bacteria-antibiotic combination

Result :


Growth
Antimicrobial disk
ATB 1
ATB 2
ATB 3
ATB 4
ATB 5
Zone of inhibition (no growth)
10
20
30
40
50
Use a ruler with a handle if calipers are unavailable


Day 1
Day 3


Gen
CIZ
PIP
100
Cef


antibiotic disc
inner zone: resistant strain
black zone: intermediate susceptibility
outer zone: susceptible strain

❑ Why should we use Muller Hinton agar ?

Mueller and Hinton developed Mueller Hinton Agar (MHA) in 1941 for the isolation of pathogenic *Neisseria* species. Nowadays, it is more commonly used for the routine susceptibility testing of non-fastidious microorganism by the **Kirby-Bauer** disk diffusion technique.

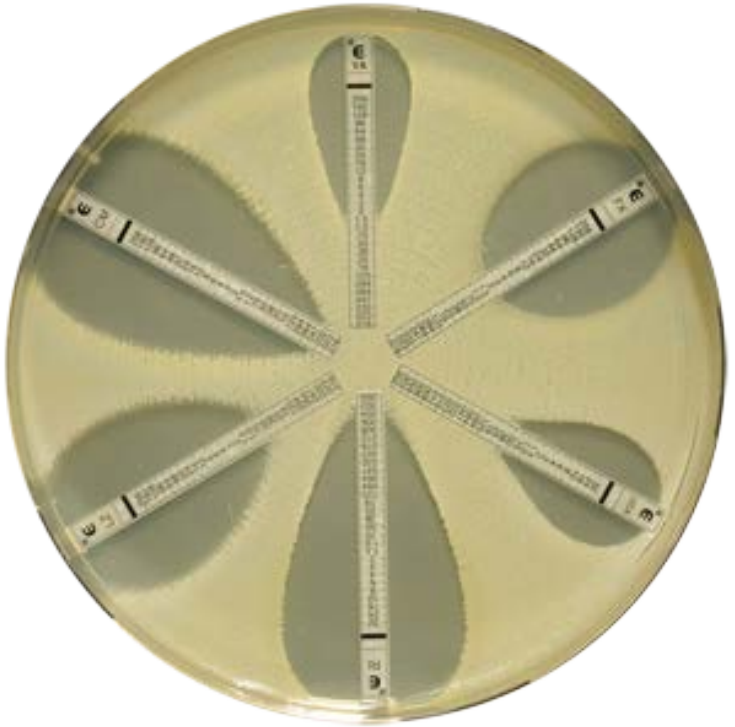
Composition of MHA/ Liter

Ingredients	Function
• Beef Extract	• provide nitrogen, vitamins, carbon, amino acids, sulphur and other essential nutrients
• Acid Hydrolysate	
• Starch	• Absorb any toxic metabolites produced • Hydrolysis yields dextrose, which serves as a source of energy
• Agar	• Solidifying agent.

3. E - test

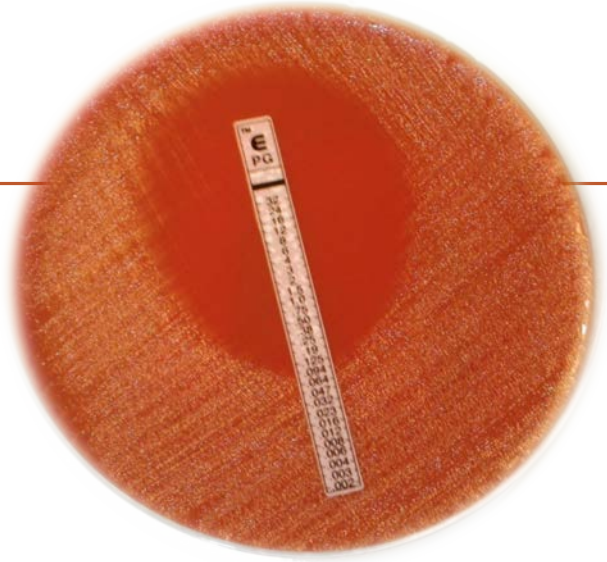
E-test is a commercially available test that utilizes a plastic test strip impregnated with a gradually decreasing concentration of a particular antibiotic.

The strip also displays a numerical scale that corresponds to the antibiotic concentration contained therein.



This method provides for a convenient quantitative test of antibiotic resistance of a clinical isolate.

However, a separate strip is needed for each antibiotic, and therefore the cost of this method can be high.

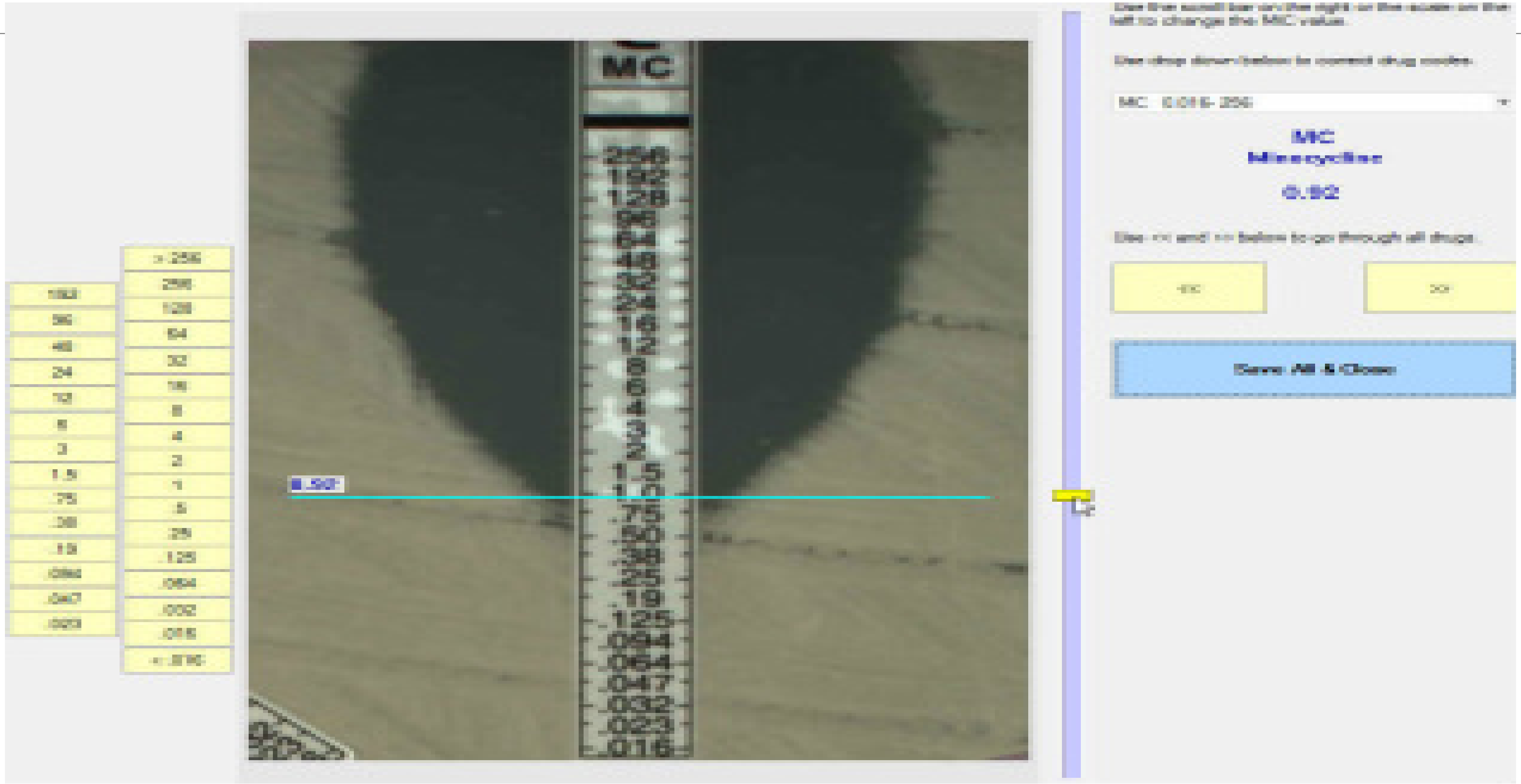


❑ Result :

Interpret results as “resistant” or “susceptible” according to the guidelines provided in the package insert

For ambiguous results, refer to the provided reading guide for :

- Organism related effects
- Drug related effects
- Resistance mechanism related effects
- Technical and handling effects


MC

Any Questions