

320 MBIO

Microbial Diagnosis

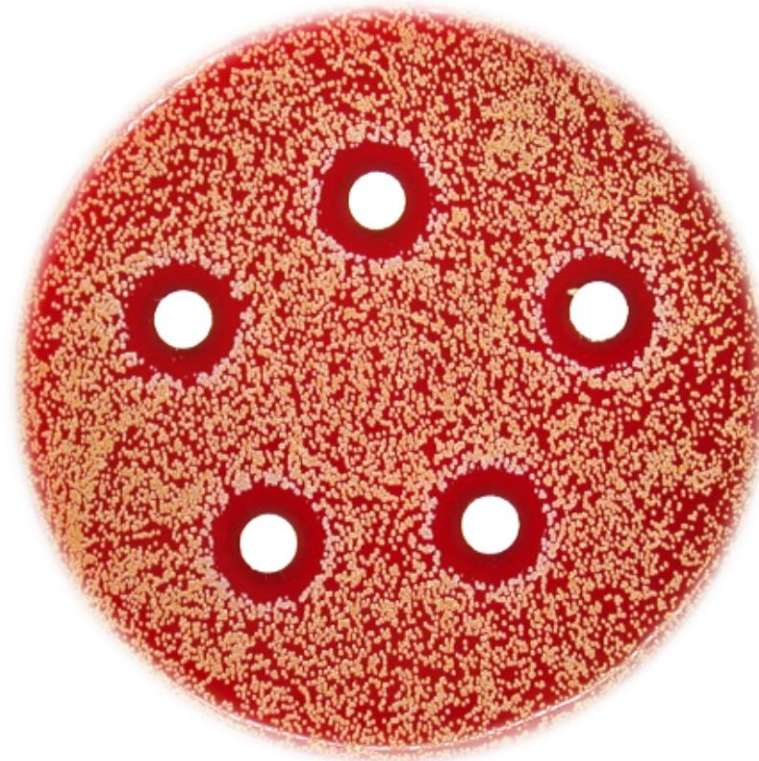
Lab 5

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2017

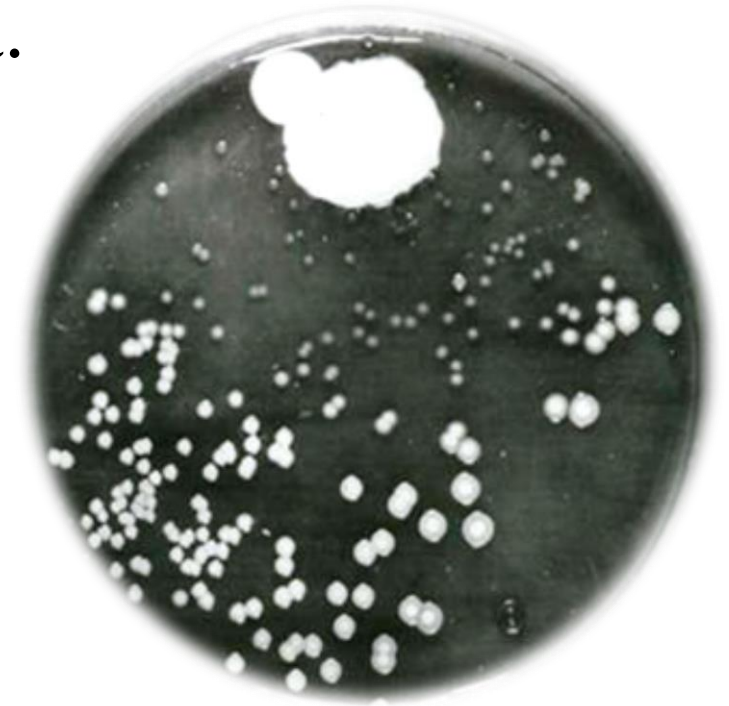
Assessing Antibiotic Effectiveness



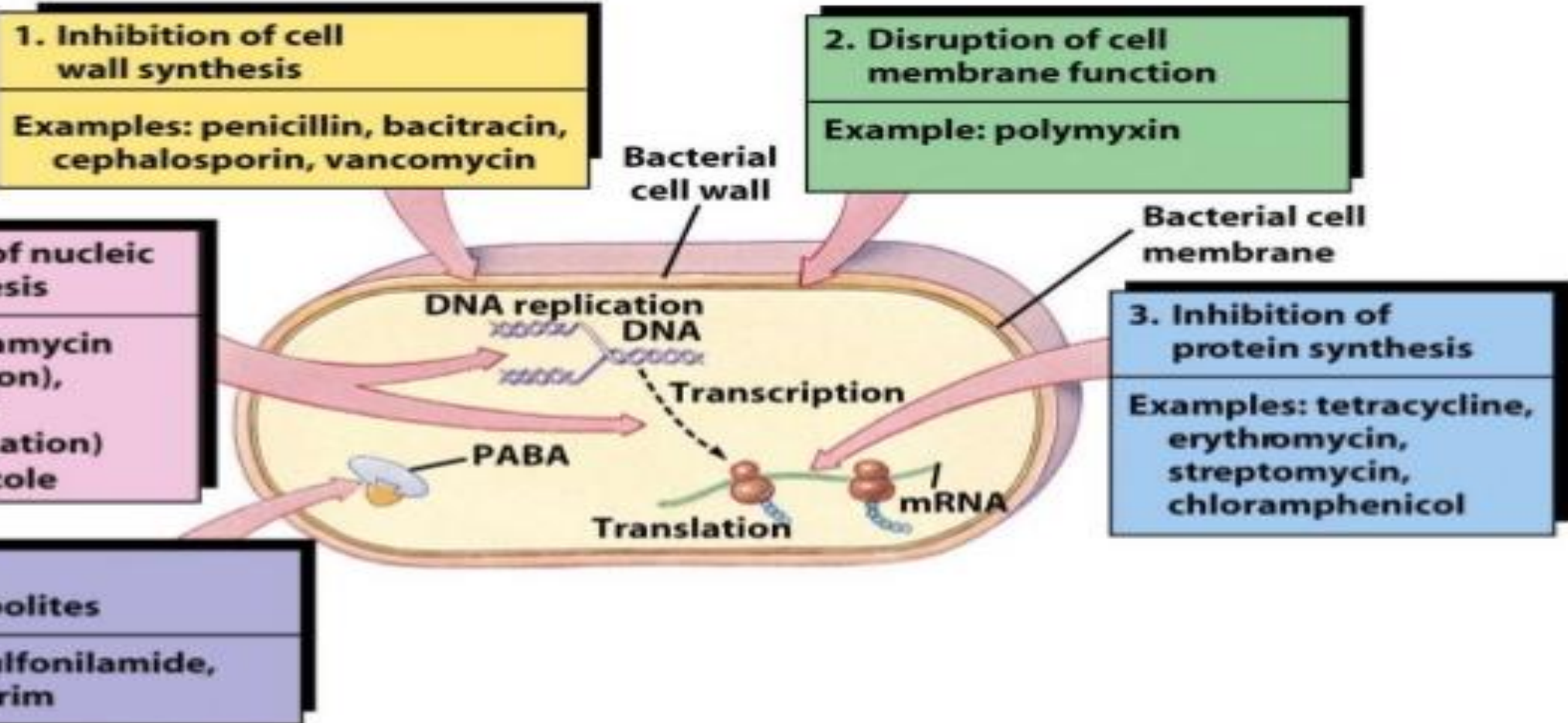
- Antibiotic have become a standard method used by physician to treat bacterial disease.
- The first antibiotic was founded by **Alexander Fleming**. It was penicillin that produced by his molds over 60 years ago.



- 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

- An active against both Gram positive and Gram negative organisms.
- **For example :** Tetracyclines

Narrow spectrum

- Have limited activity and are primarily only useful against particular species of microorganisms.
- **For example :**
 - Polymixins → Gram negative
 - Bacitracin → Gram positive

❖ Examples of Antibiotic Sensitivity Testing Methods :

1 Dilution methods

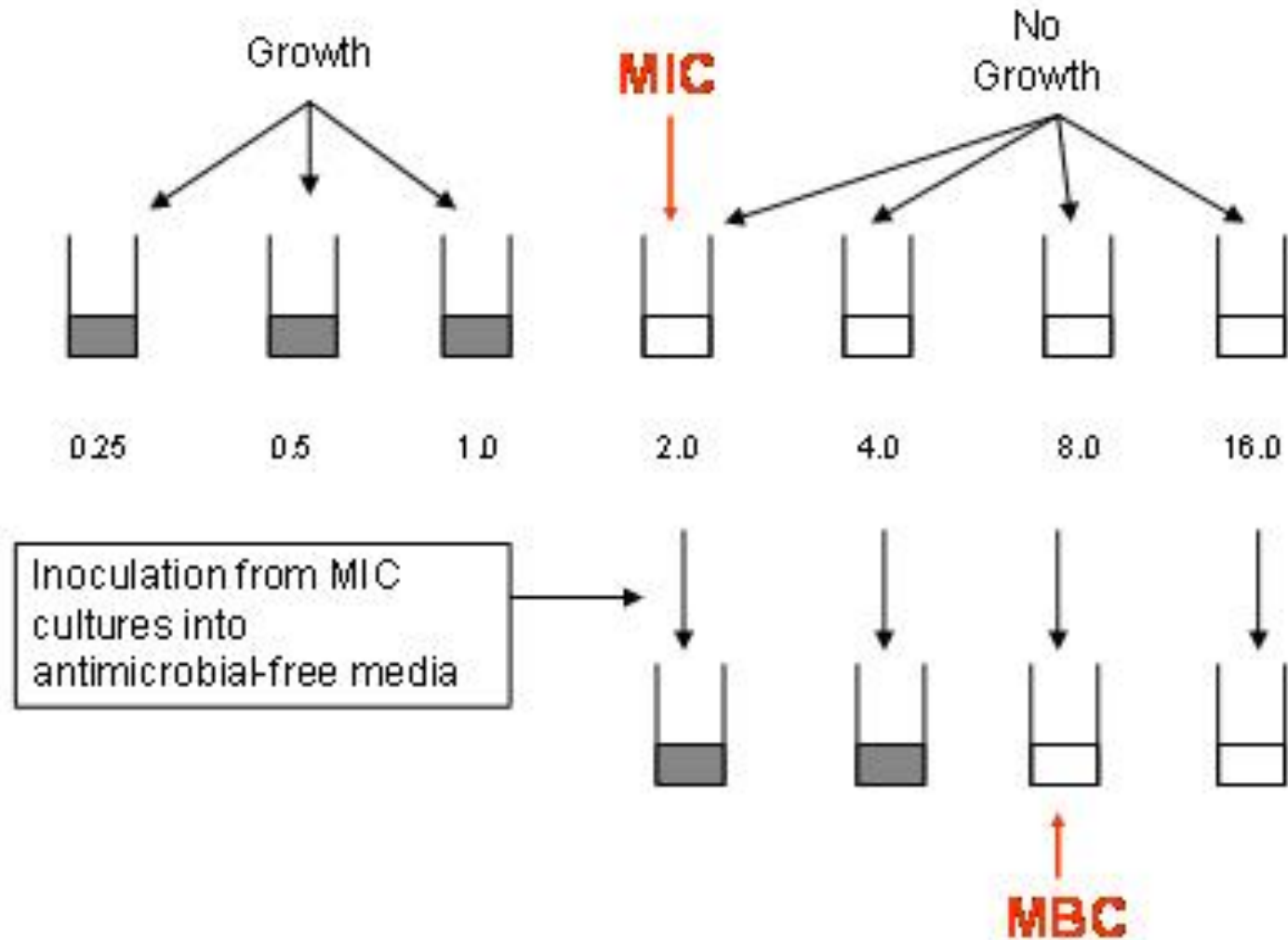
2 Disk diffusion method

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.

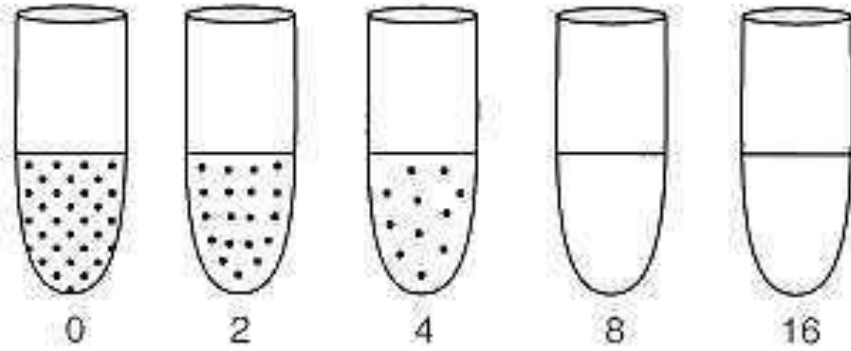
Serial Dilution Susceptibility Testing



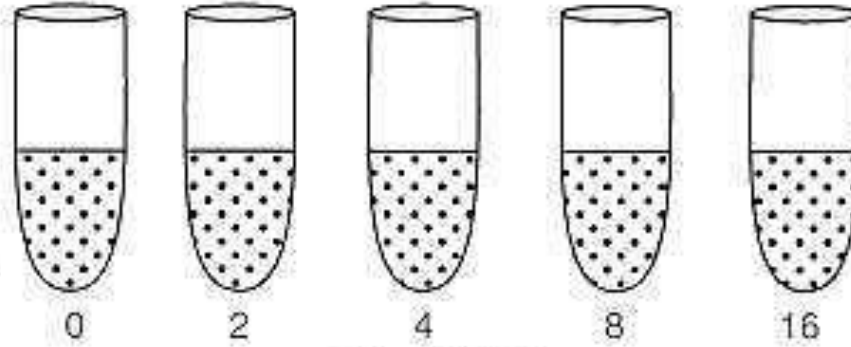
Antibiotic susceptibility tests

Minimum inhibitory concentration test

A
Susceptible
organism

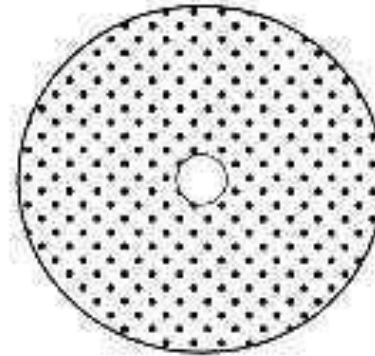
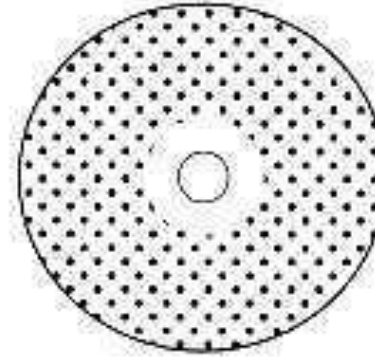


B
Resistant
organism



$\mu\text{g/ml}$ antibiotic

Disk diffusion test



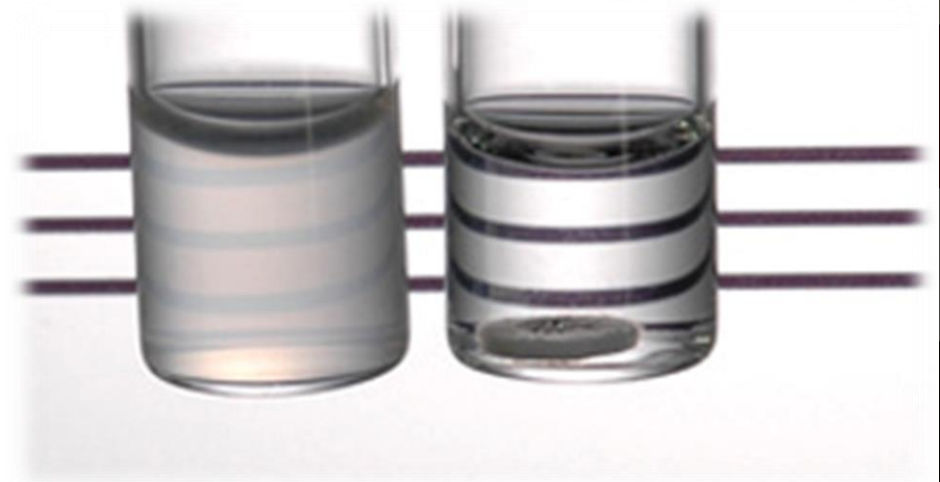
10 μg antibiotic in discs

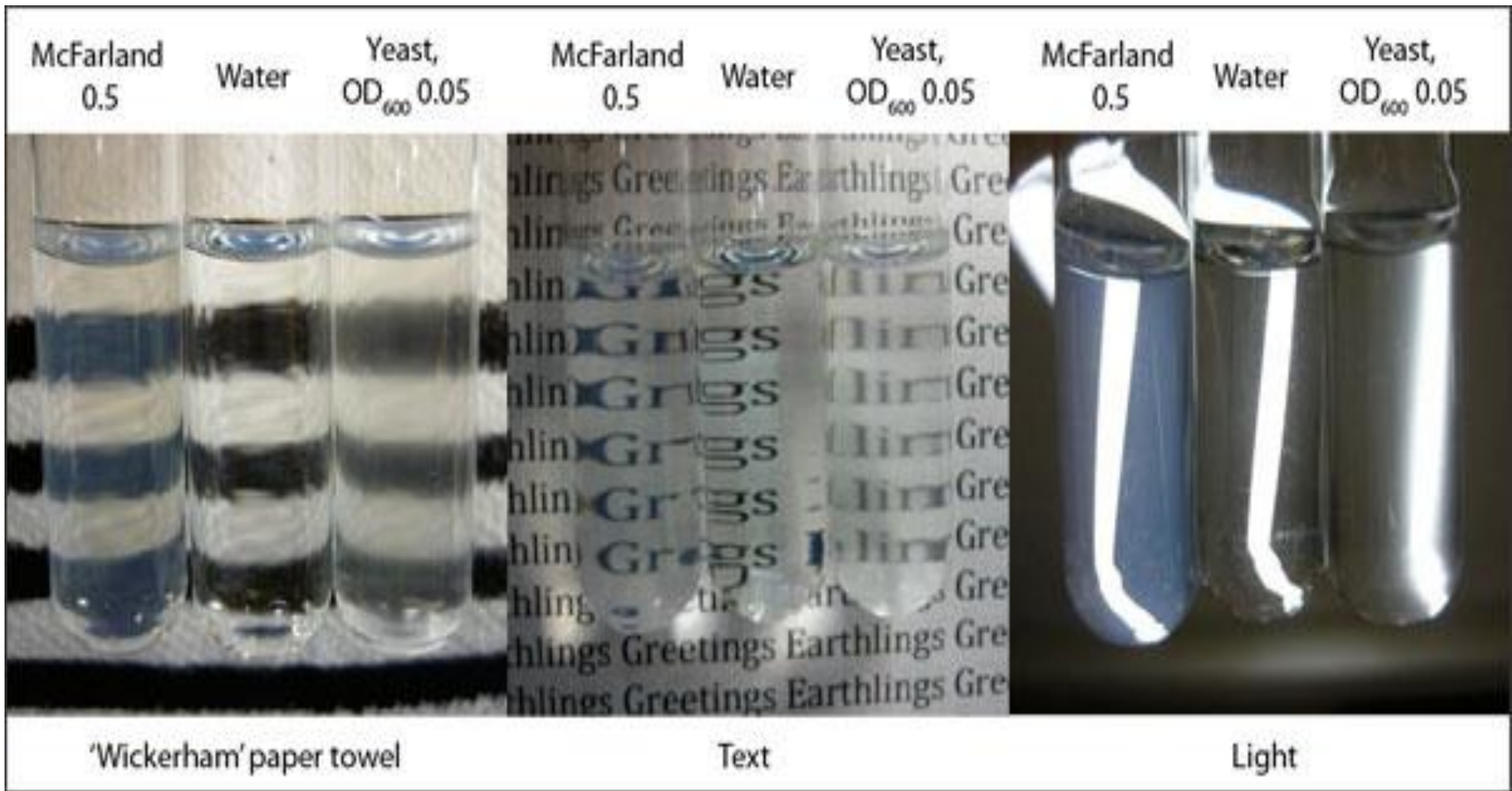
2. Disk Diffusion Method (Kirby Bauer Test):

- 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

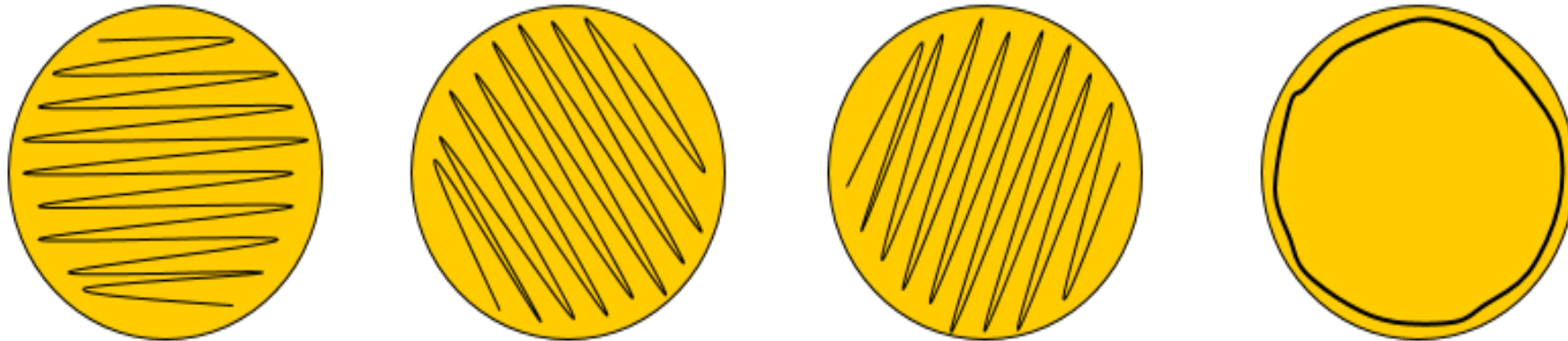
□ Procedures :

- 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**.



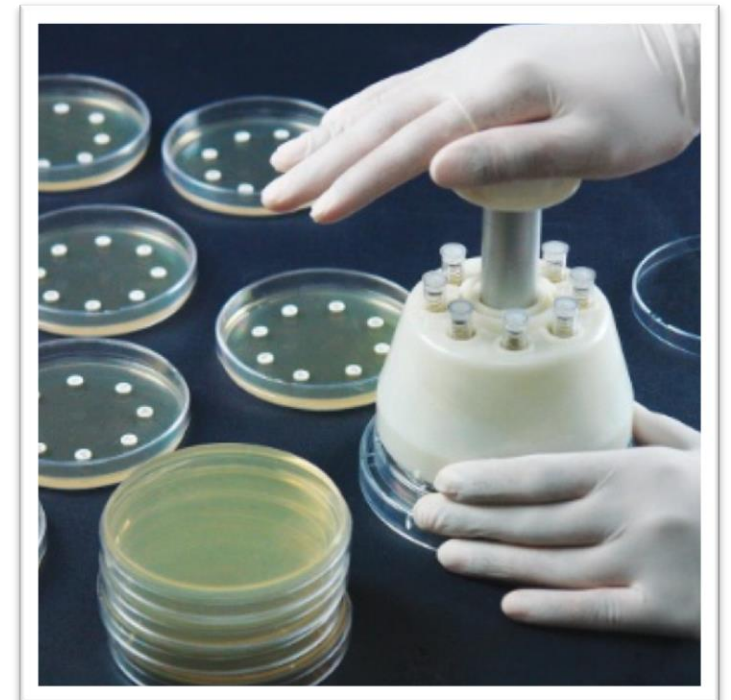


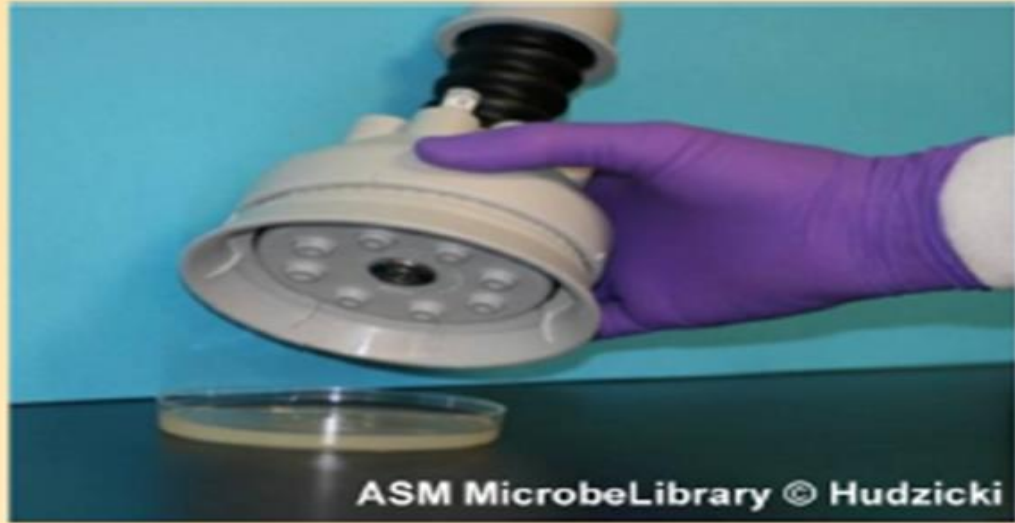
- Within 15 minutes of adjusting the turbidity, dip a sterile cotton swab into the sample.
- Streak a lawn of bacteria on Mueller-Hinton agar



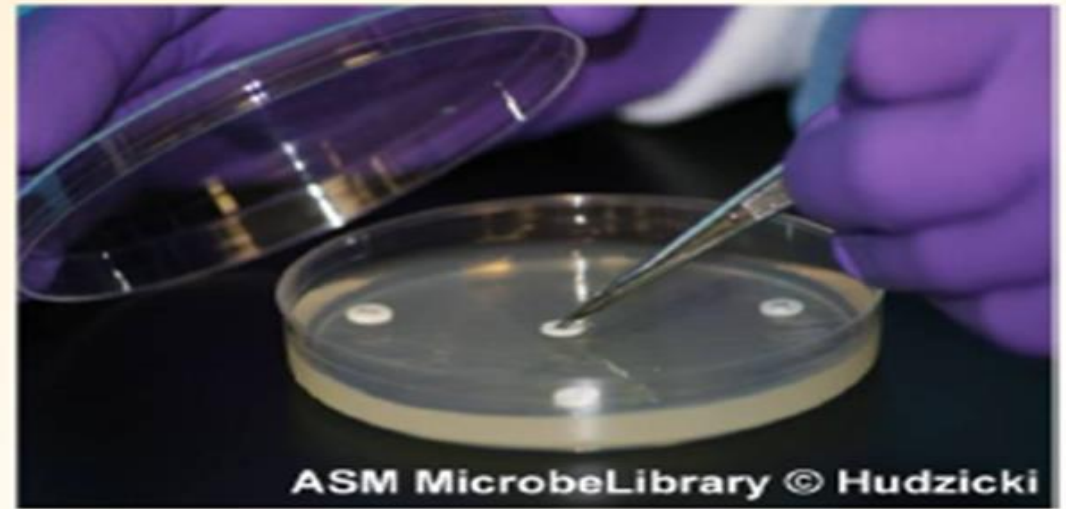
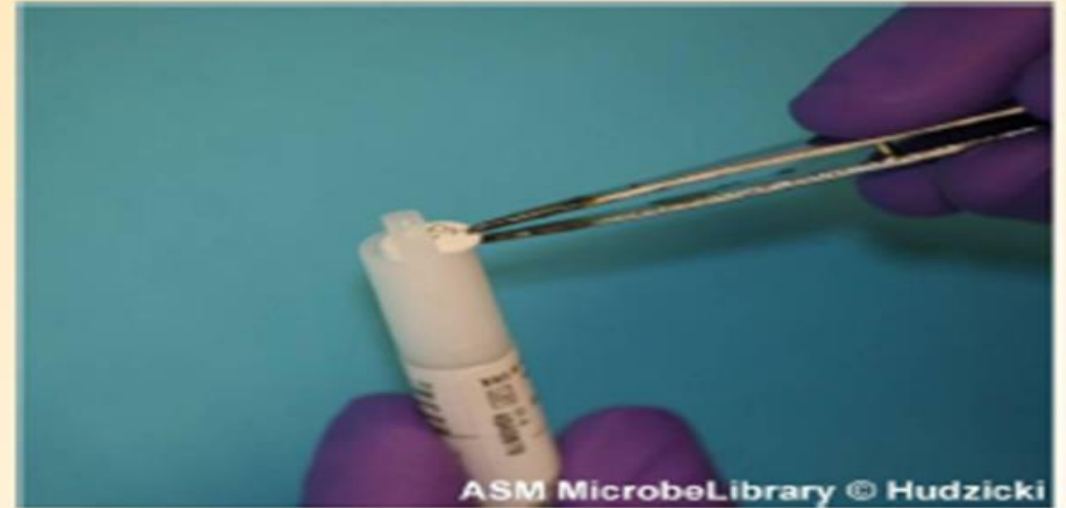
- Leave the lid agar for 3-5 minutes (no more than 15 minutes) to allow plate to dry.

- 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.





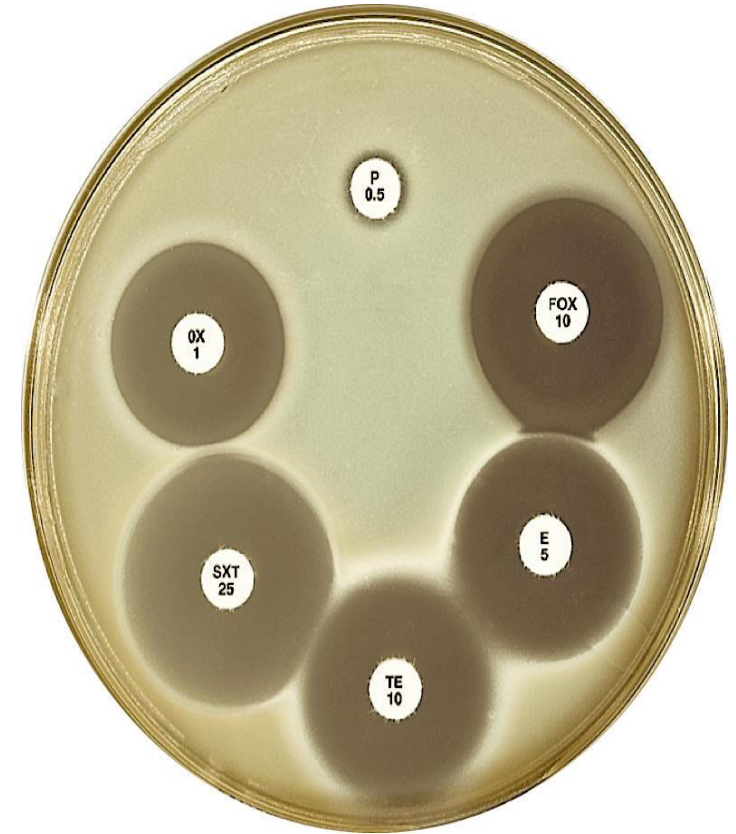
**placement of antibiotic disks using
an automated disk dispenser**



placement of antibiotic disks using forceps

□ Result :

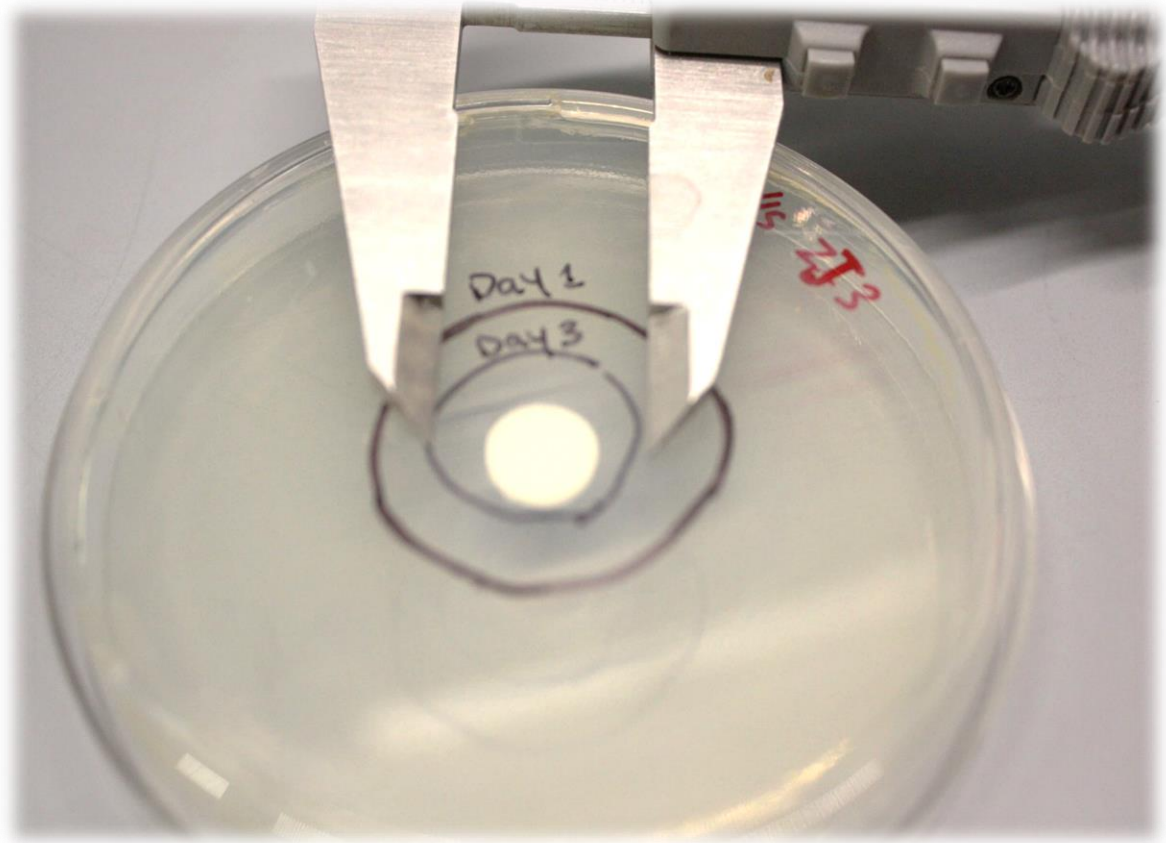
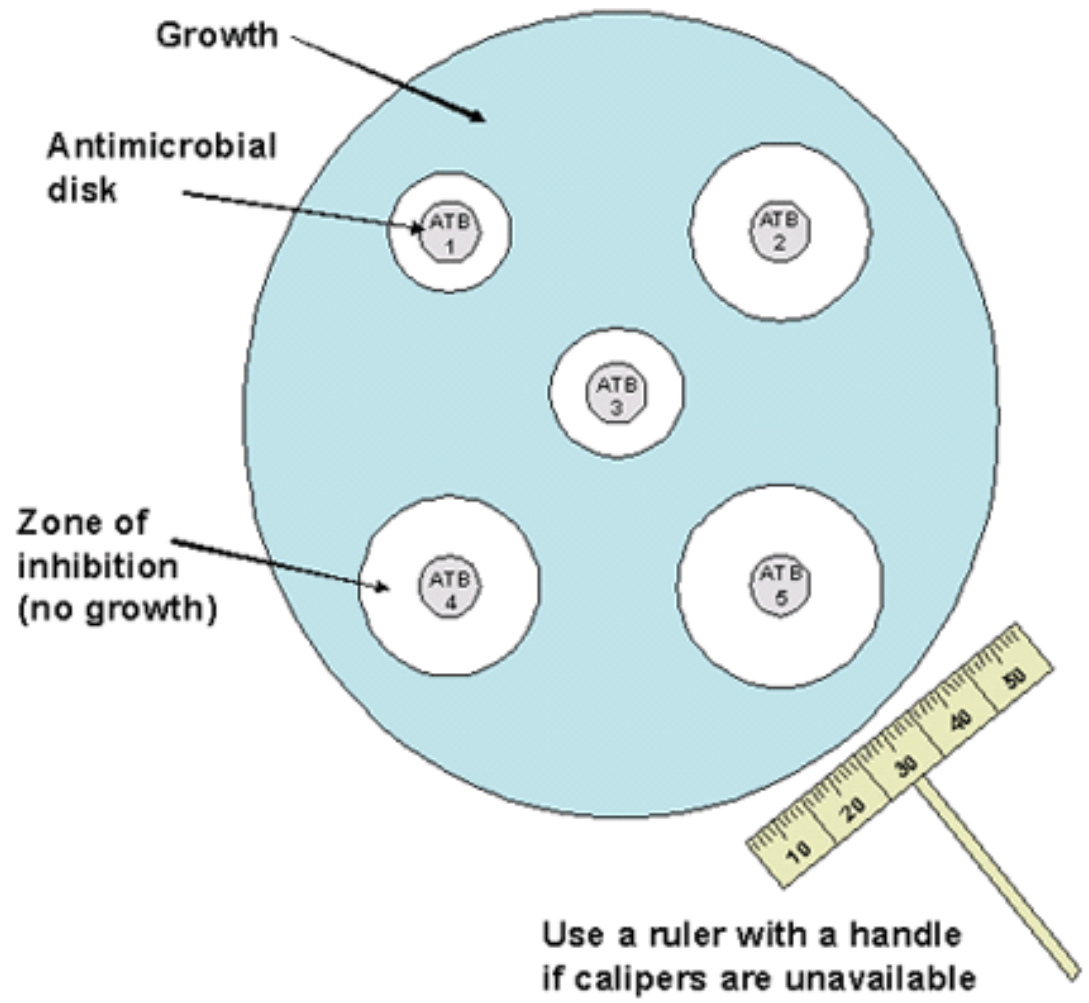
- Antibiotics diffuse out onto the agar.
- Concentration of antibiotics decrease as they diffuse further away from the disks
- After incubation, observe for a clearing on the bacterial lawn (zone of inhibition)



□ 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 :





antibiotic disc

inner zone: resistant strain

black zone: intermediate
susceptibility

outer zone: susceptible strain



□ Why should we use Muller Hinton agar ?

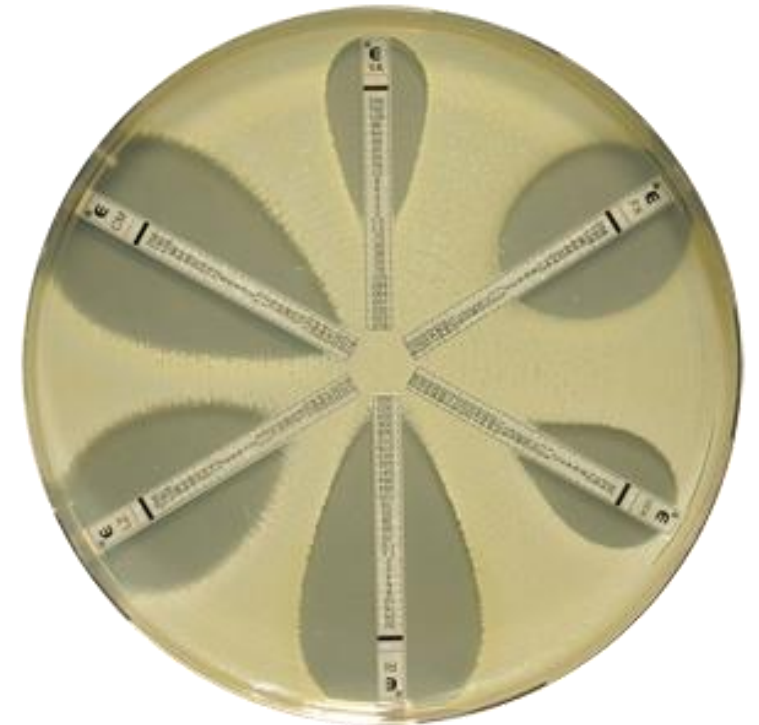
- Mueller and Hinton developed Mueller Hinton Agar (MHA) in 1941 for the isolation of pathogenic *Neisseria* species. Nowadays, it is more commonly used for the routine susceptibility testing of non-fastidious microorganism by the **Kirby-Bauer** disk diffusion technique.

□ Composition of MHA/ Liter

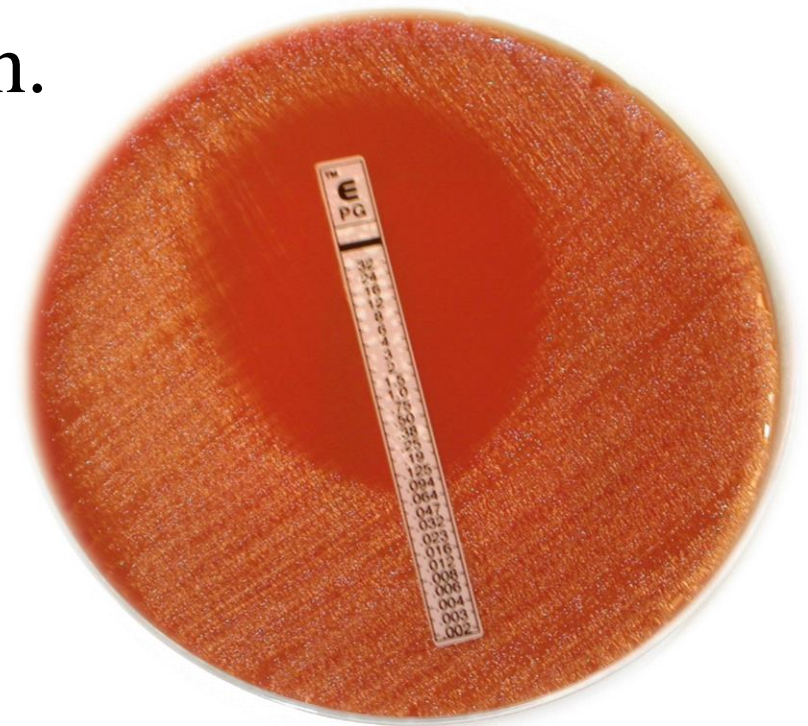
Ingredients	Function
<ul style="list-style-type: none"> • Beef Extract 	<ul style="list-style-type: none"> • provide nitrogen, vitamins, carbon, amino acids, sulphur and other essential nutrients
<ul style="list-style-type: none"> • Acid Hydrolysate 	
<ul style="list-style-type: none"> • Starch 	<ul style="list-style-type: none"> • Absorb any toxic metabolites produced • Hydrolysis yields dextrose, which serves as a source of energy
<ul style="list-style-type: none"> • Agar 	<ul style="list-style-type: none"> • 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

See the sound bar on the right or the scale on the left to change the MIC value.

See drug description below to correct drug name.

MC: 0.016-256

MC
Minocycline
0.50

See in and in below to go through all drugs.

OK Cancel

Save All & Close

192	> 256
96	256
48	128
24	64
12	32
6	16
3	8
1.5	4
.75	2
.38	1
.19	.5
.096	.25
.047	.125
.023	.064
	.032
	.016
	< 0.16

Any Questions

