

320 MBIO

Microbial Diagnosis

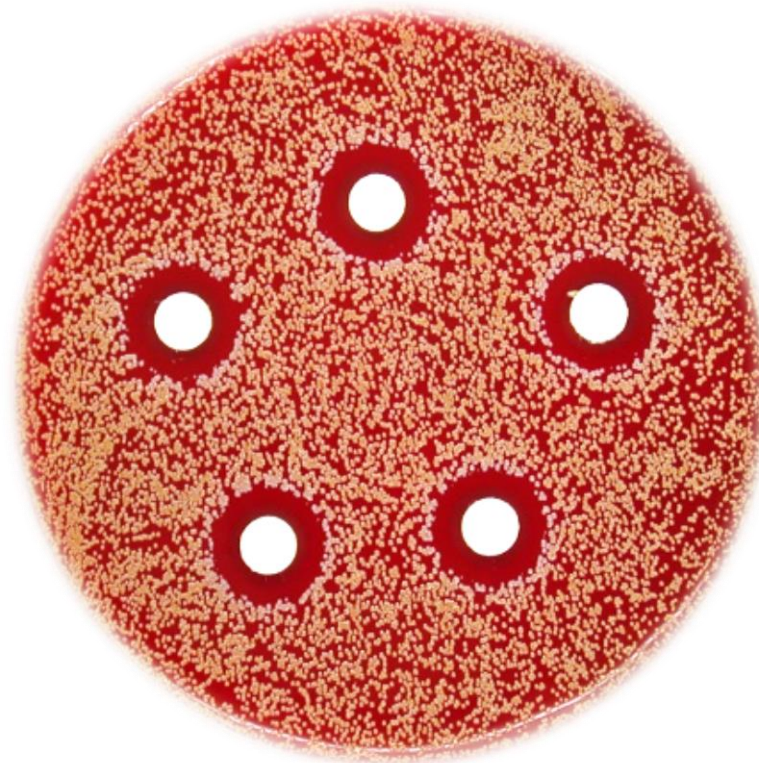
Lab 5

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2017

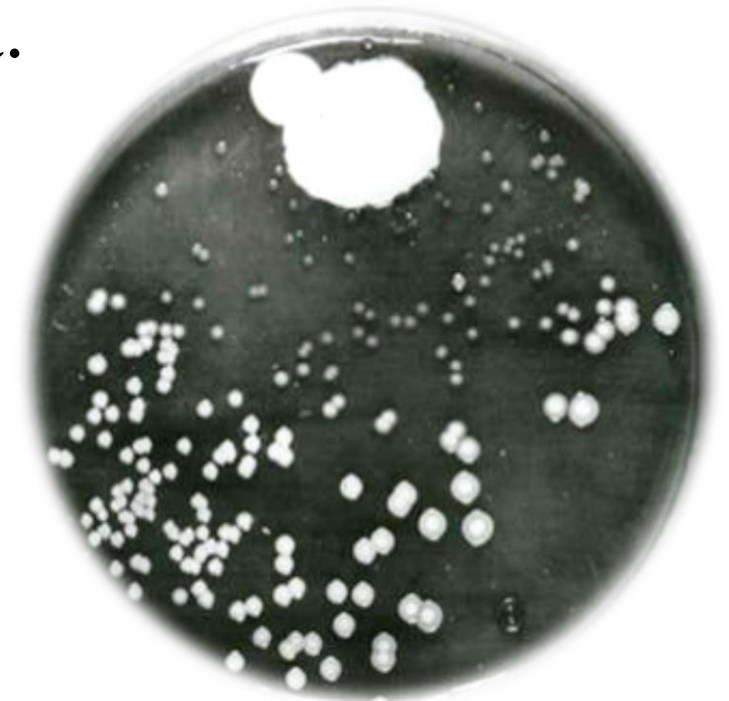
Assessing Antibiotic Effectiveness



- 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

1. Inhibition of cell wall synthesis

Examples: penicillin, bacitracin, cephalosporin, vancomycin

2. Disruption of cell membrane function

Example: polymyxin

4. Inhibition of nucleic acid synthesis

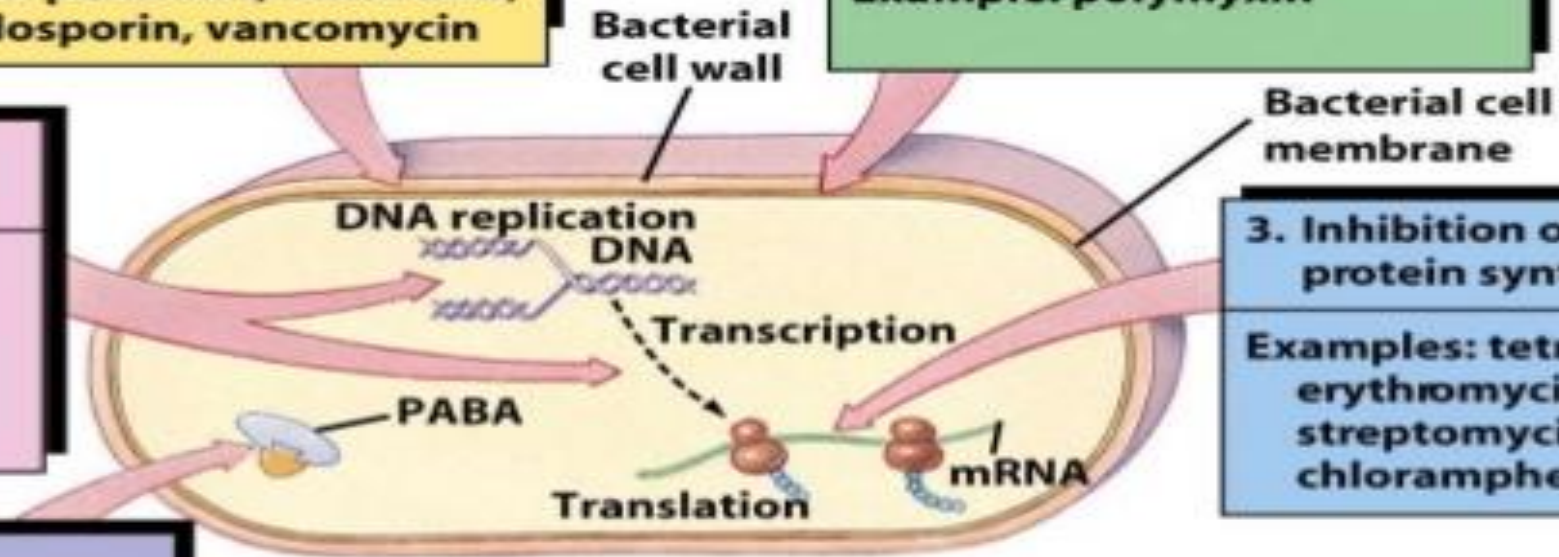
Examples: rifamycin (transcription), quinolones (DNA replication) metronidazole

5. Action as antimetabolites

Examples: sulfonilamide, trimethoprim

3. Inhibition of protein synthesis

Examples: tetracycline, erythromycin, streptomycin, chloramphenicol



- 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**

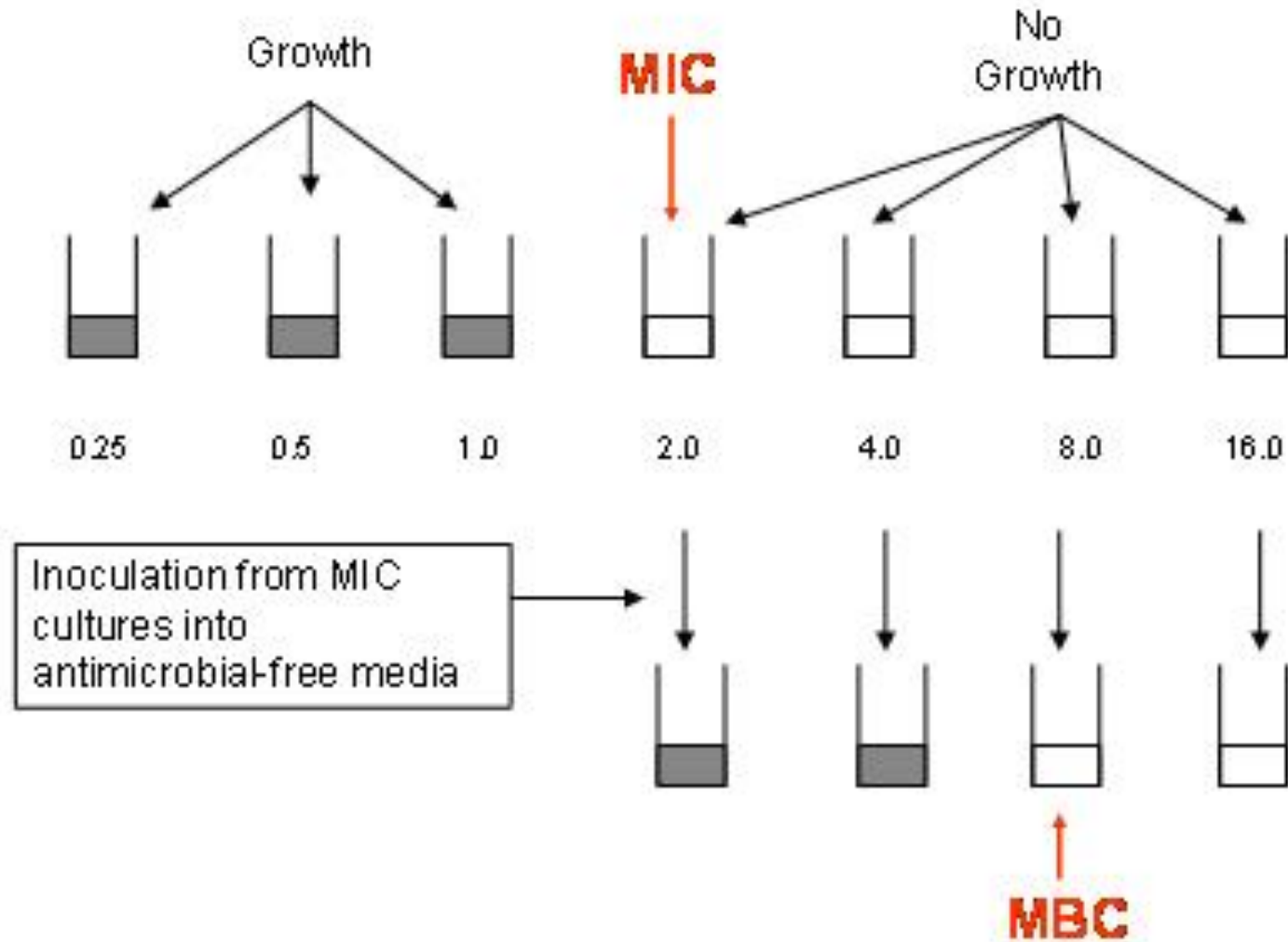
2 **Disk diffusion method**

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.

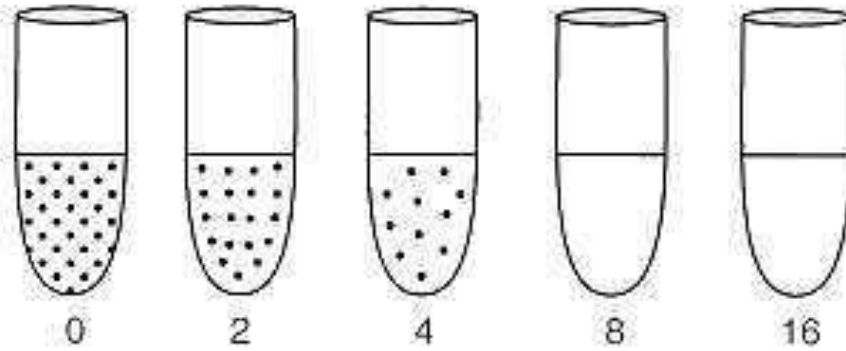
Serial Dilution Susceptibility Testing



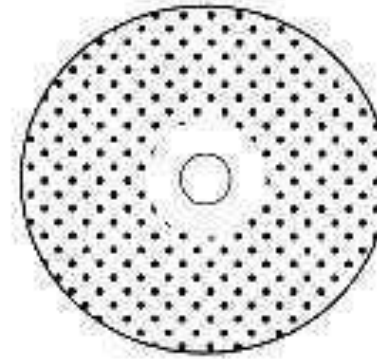
Antibiotic susceptibility tests

Minimum inhibitory concentration test

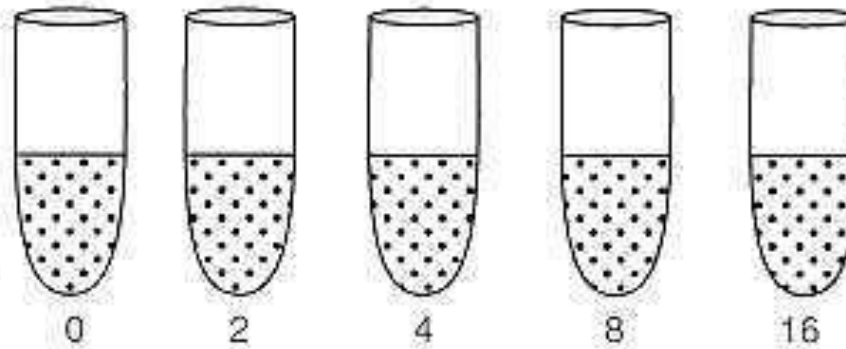
A
Susceptible
organism



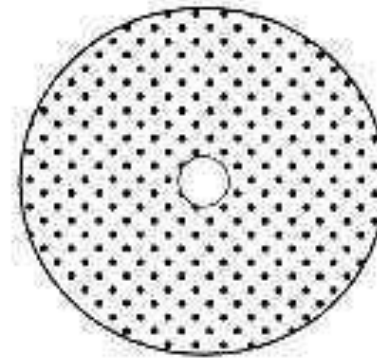
Disk diffusion test



B
Resistant
organism



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



10 μg antibiotic in discs

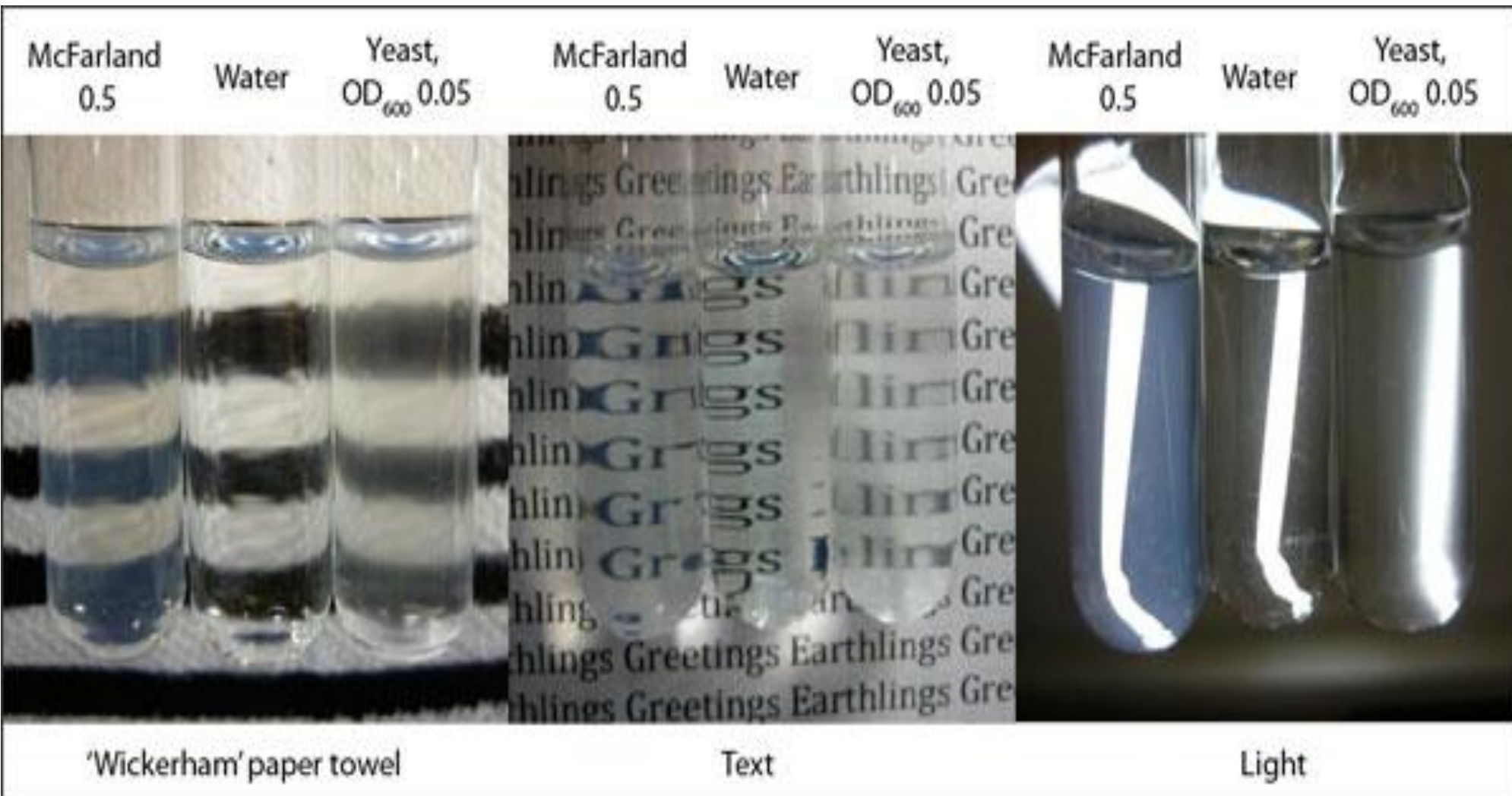
2. Disk Diffusion Method (Kirby Bauer Test):

- 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

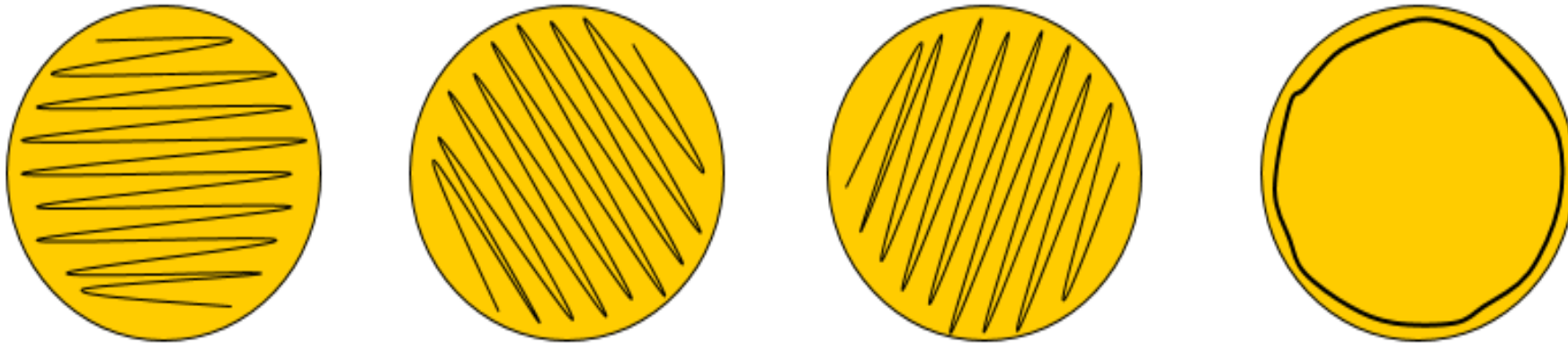
□ Procedures :

- 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.





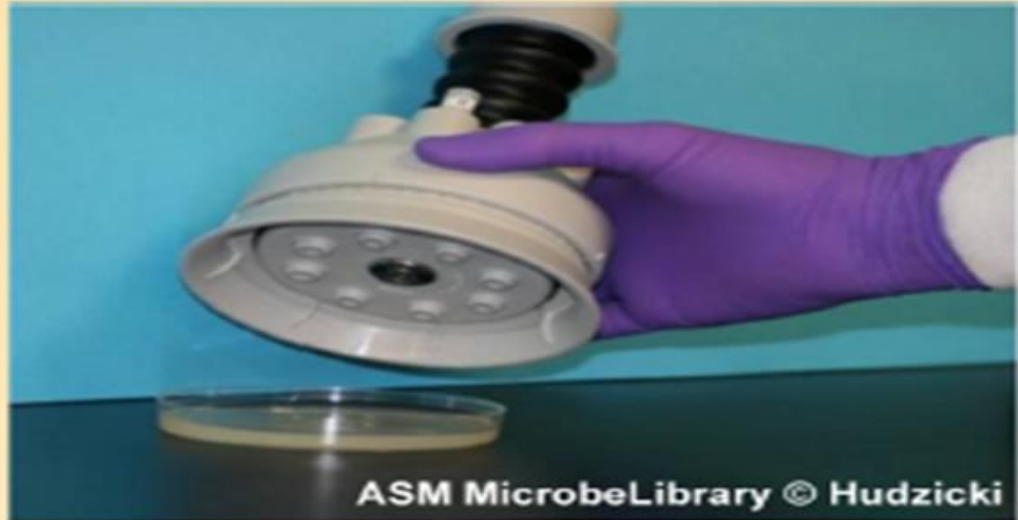
- Within 15 minutes of adjusting the turbidity, dip a sterile cotton swab into the sample.
- Streak a lawn of bacteria on Mueller-Hinton agar



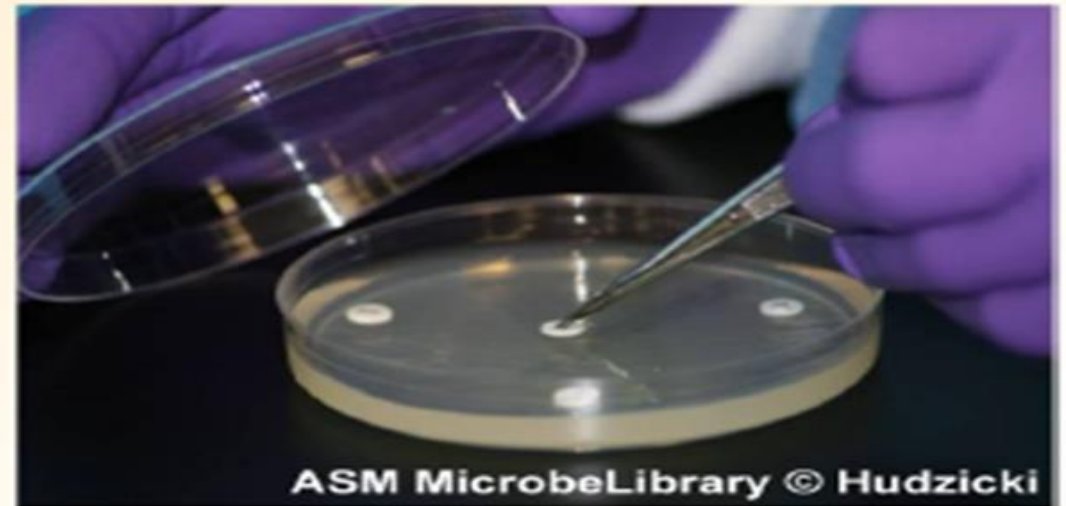
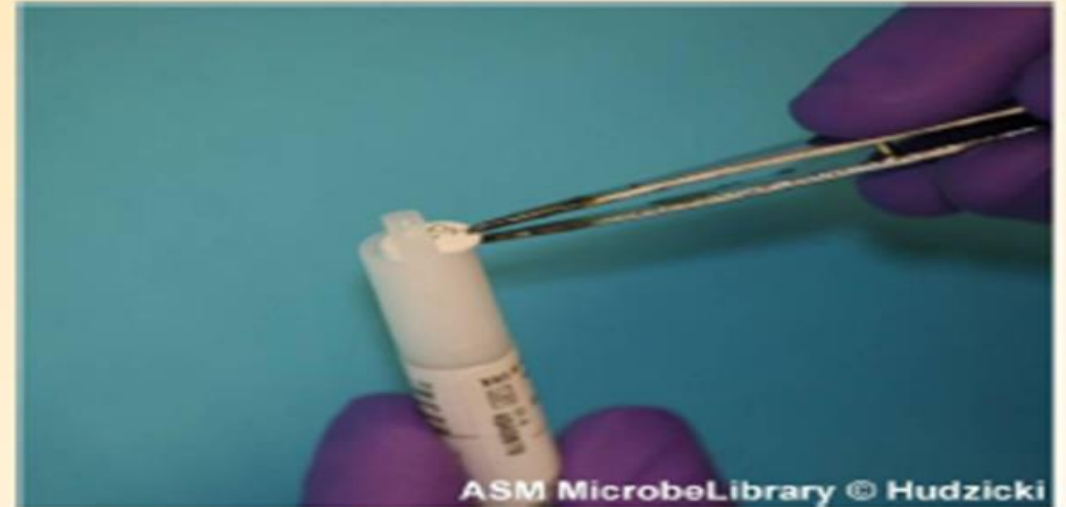
- Leave the lid agar for 3-5 minutes (no more than 15 minutes) to allow plate to dry.

- 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.





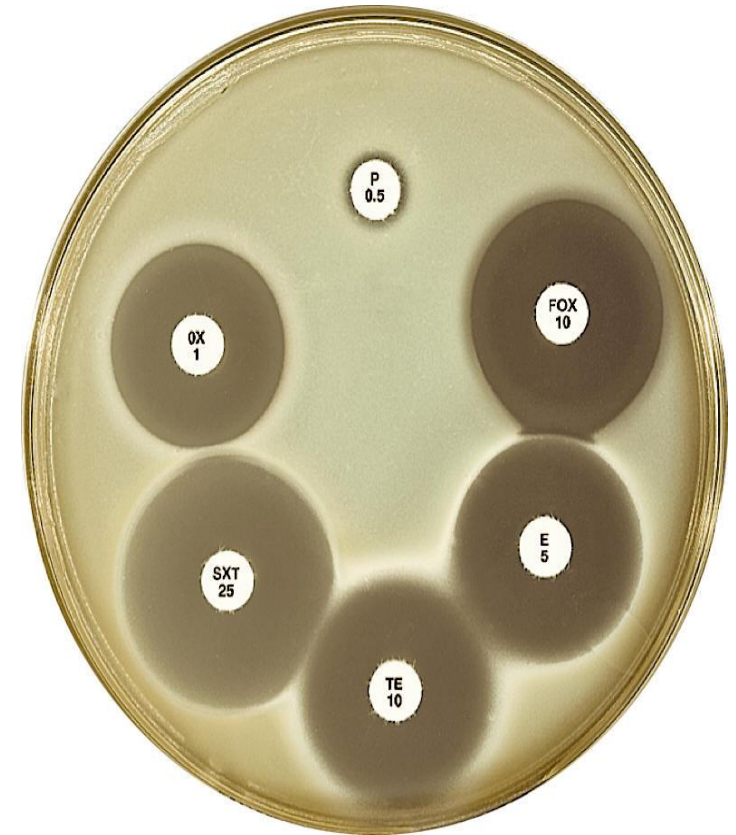
**placement of antibiotic disks using
an automated disk dispenser**



placement of antibiotic disks using forceps

□ Result :

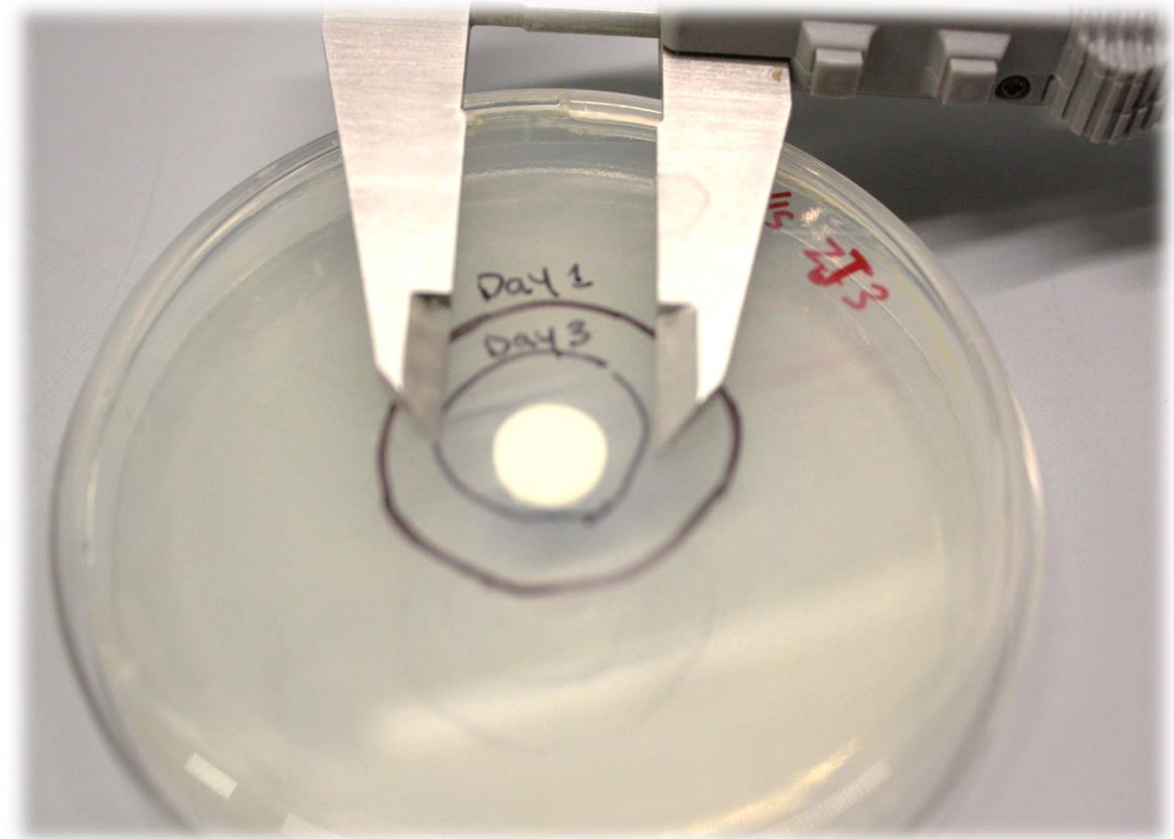
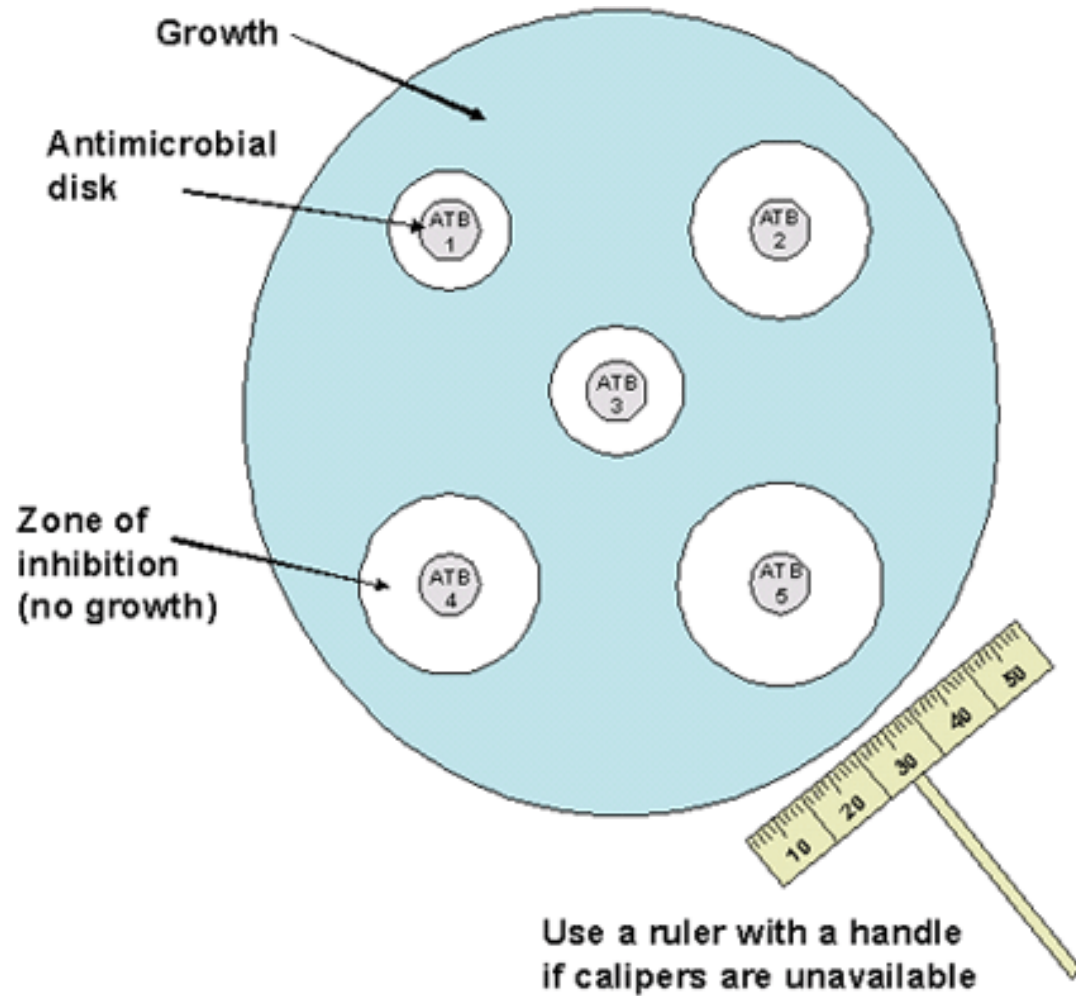
- Antibiotics diffuse out onto the agar.
- Concentration of antibiotics decrease as they diffuse further away from the disks
- After incubation, observe for a clearing on the bacterial lawn (zone of inhibition)



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

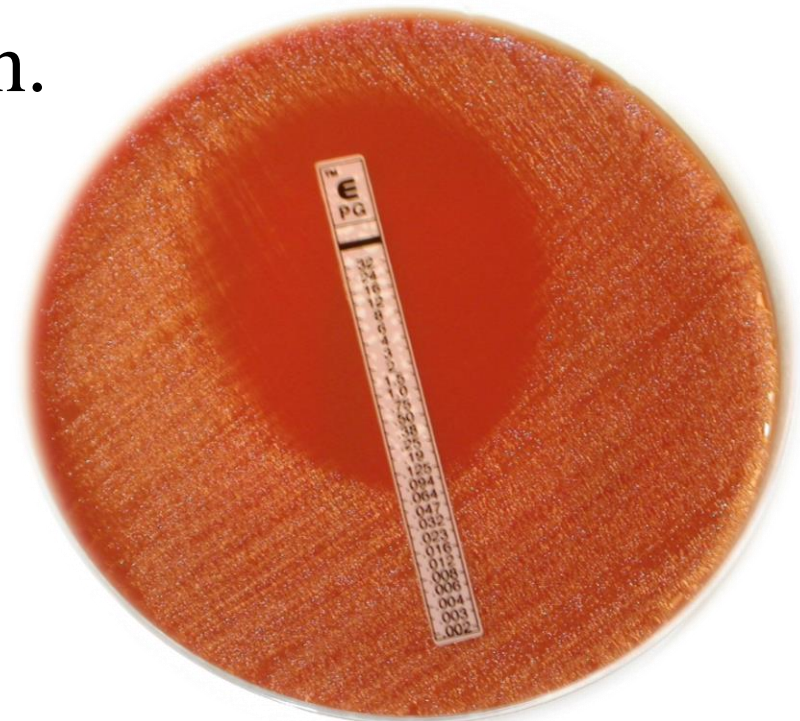
Ingredients	Function
<ul style="list-style-type: none"> Beef Extract Acid Hydrolysate 	<ul style="list-style-type: none"> provide nitrogen, vitamins, carbon, amino acids, sulphur and other essential nutrients
<ul style="list-style-type: none"> Starch 	<ul style="list-style-type: none"> Absorb any toxic 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.

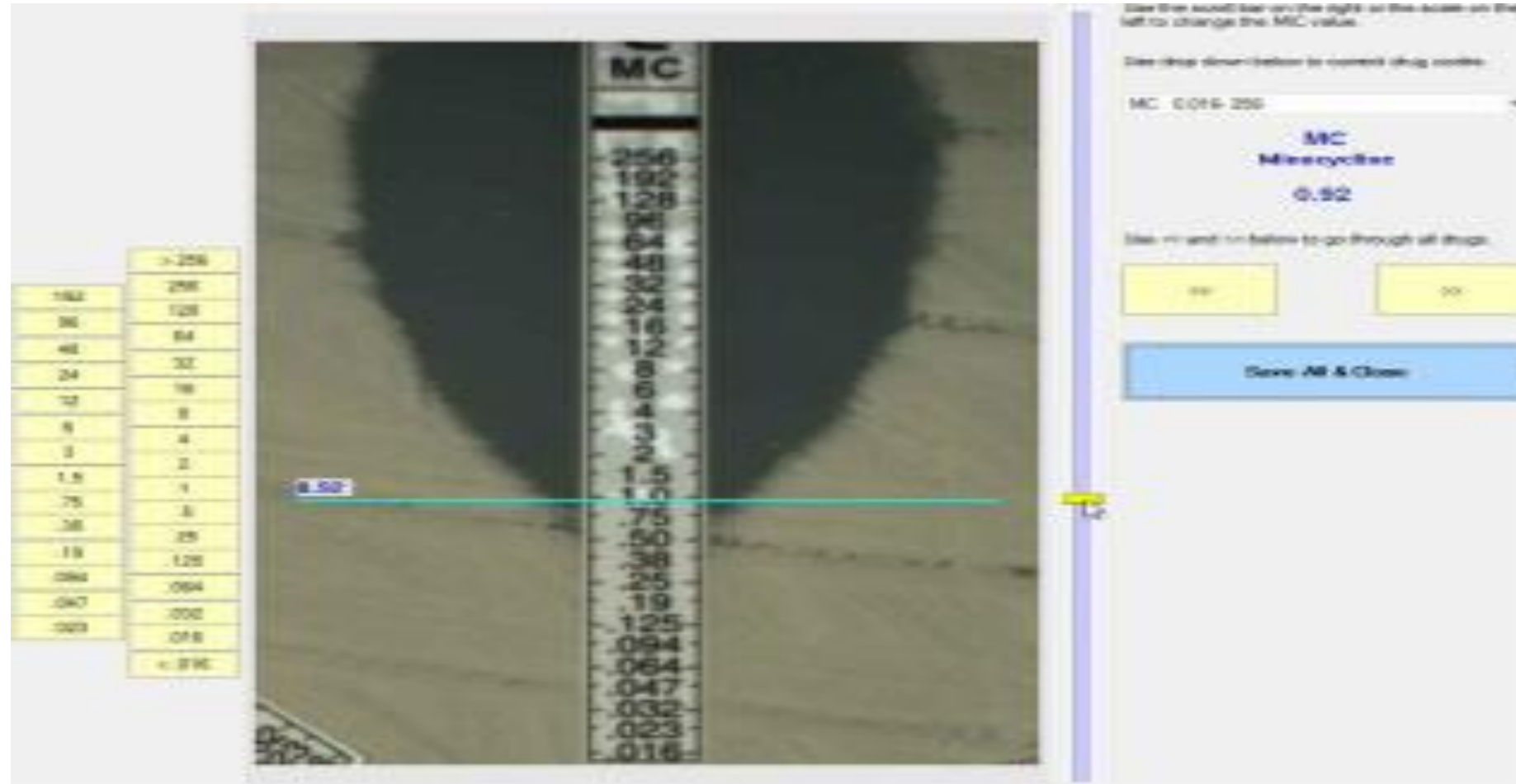


- This method provides for a convenient quantitative test of antibiotic resistance of a clinical isolate.
- However, a separate strip is needed for each antibiotic, and therefore the cost of this method can be high.



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





Any Questions

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