Antibiotic sensitivity Testing

Antimicrobial Susceptibility Testing for Bacteriology Laboratories

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EMCParadigm PUBLISHING

- **Antibiotic**: Chemical produced by a microorganism that kills or inhibits the growth of another microorganism

- **Types of antibiotic:-**
  - **Narrow spectrum antibiotic**
    active against either Gram +ve bacteria only or gram –ve bacteria only

  - **Broad spectrum antibiotic**
    active against both Gram's +ve and -ve bacteria

Antibiotic Sensitivity Testing

- **Antibiotic sensitivity testing is used to determine the susceptibility of the microorganism to various antimicrobial agents.**

Sensitivity testing techniques:

Disc Diffusion Method.

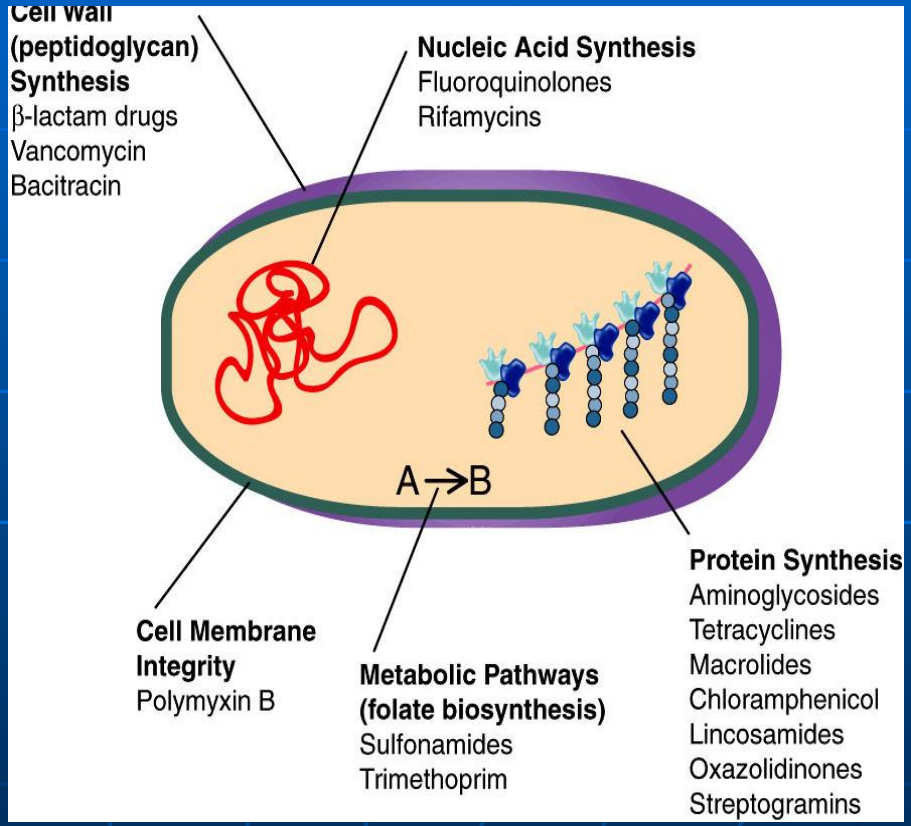
Serial Dilution Method

MIC (Minimum inhibitory concentration).

MBC

MECHANISMS OF ACTION OF ANTIBACTERIAL DRUGS

- Mechanism of action include:
  - Inhibition of cell wall synthesis
  - Inhibition of protein synthesis
  - Inhibition of nucleic acid synthesis
  - Inhibition of metabolic pathways
  - Interference with cell membrane integrity



Classification of antibiotics based on mode of action

- Inhibit Cell synthesis
- Penicillin (P) Bacitracin (Ba) –Vancomycin (Va)
- Inhibit Protein synthesis
- Erythromycin (E) - Gentamycin (GN)
- Inhibit nucleic acids
- DNA/RNA Rifampicin (RF) –Ciprofloxacin (CIP)
- Inhibit of selective permeability of cell membrane
- Polymyxins
- Inhibit bacterial metabolism
- SXT) sulphamethoxazole/trimethoprin(

Routine Susceptibility Tests

- **Disk diffusion**
- (Kirby Bauer)
- (Modified Stock Technique)

- **Broth micro-dilution MIC / MBC**

- **Etest**

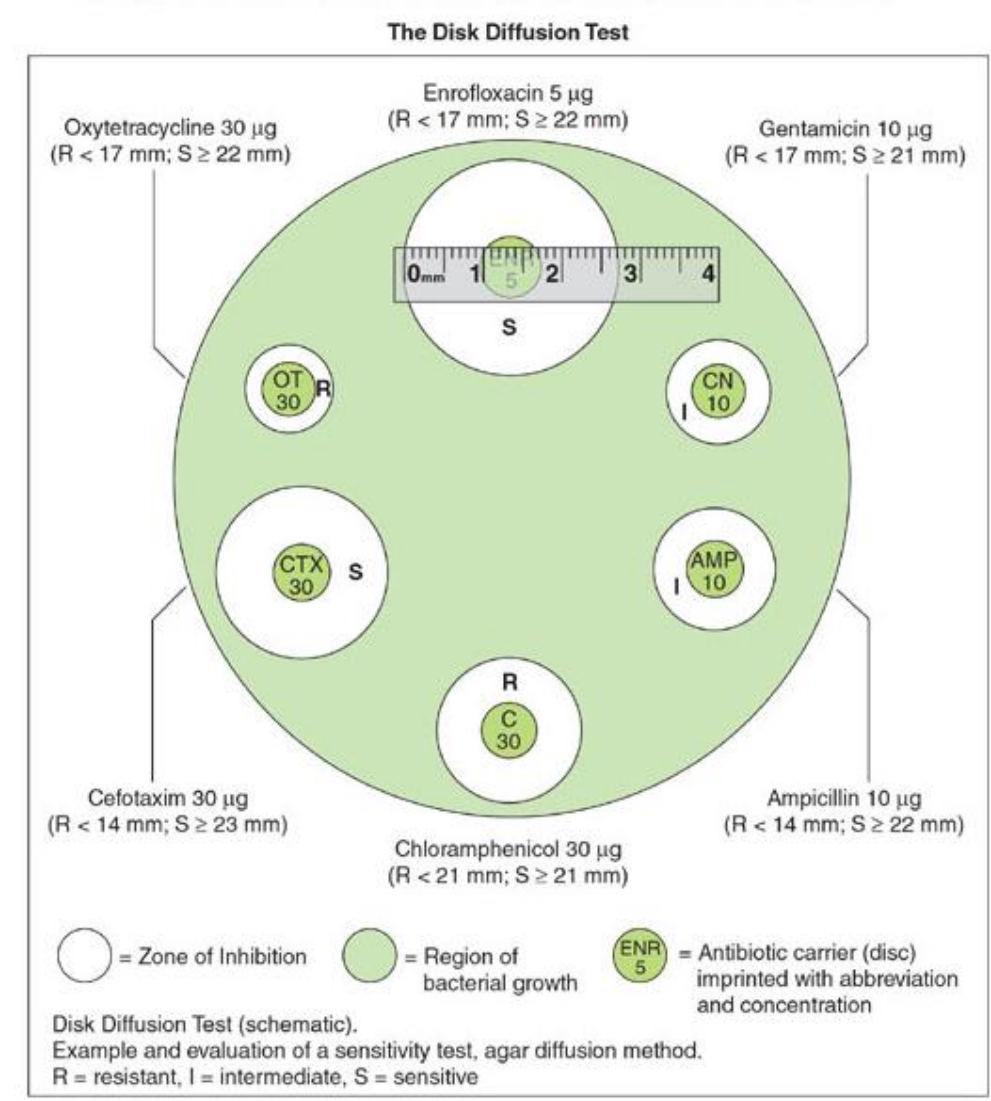
Disc Diffusion Method

- **The effectiveness of an antibiotic in this technique is based on the size of the zone of inhibition that surrounds a disc that has been impregnated with a specific concentration of the agent.**

- ***Advantages:***
  - ✓ **Rapid**
  - ✓ **Accurate**
  - ✓ **Inexpensive**

ANTIMICROBIAL SUSCEPTIBILITY TESTING

- Probably the most widely used testing method is the disk-diffusion method, also known as the Kirby-Bauer test.



- **The recommended medium →**

  Mueller Hinton agar

The inoculum:

The turbidity of a broth culture / saline suspension of the test organism has to match a defined standard

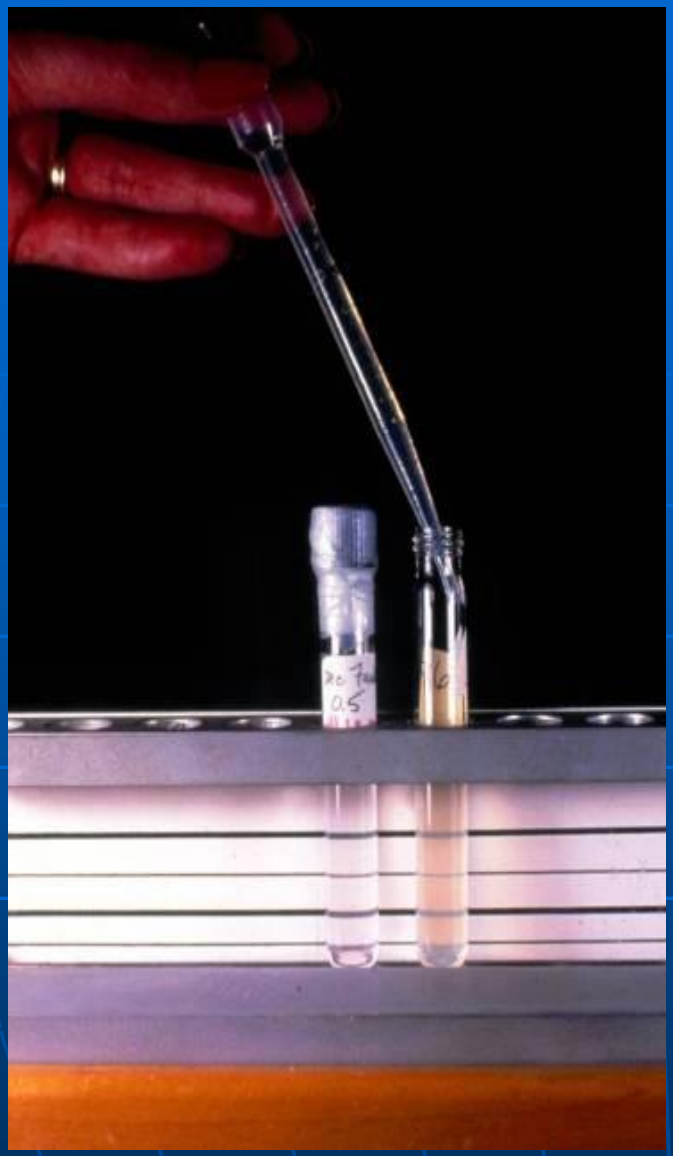
“0.5 McFarland” (a barium sulphate standard)

Disc Diffusion method

- ***Materials:***
  - **Cultures of bacteria**
  - **Filter paper disc**
  - **Antibiotic solutions (Vancomycin, Augmentin, Ceftazidime, Azactam)**
  - **20 ml melted nutrient agar**
  - **Petri-dish**
  - **Sterile 1ml pipette**

Select colonies

Prepare inoculum suspension



Mix well

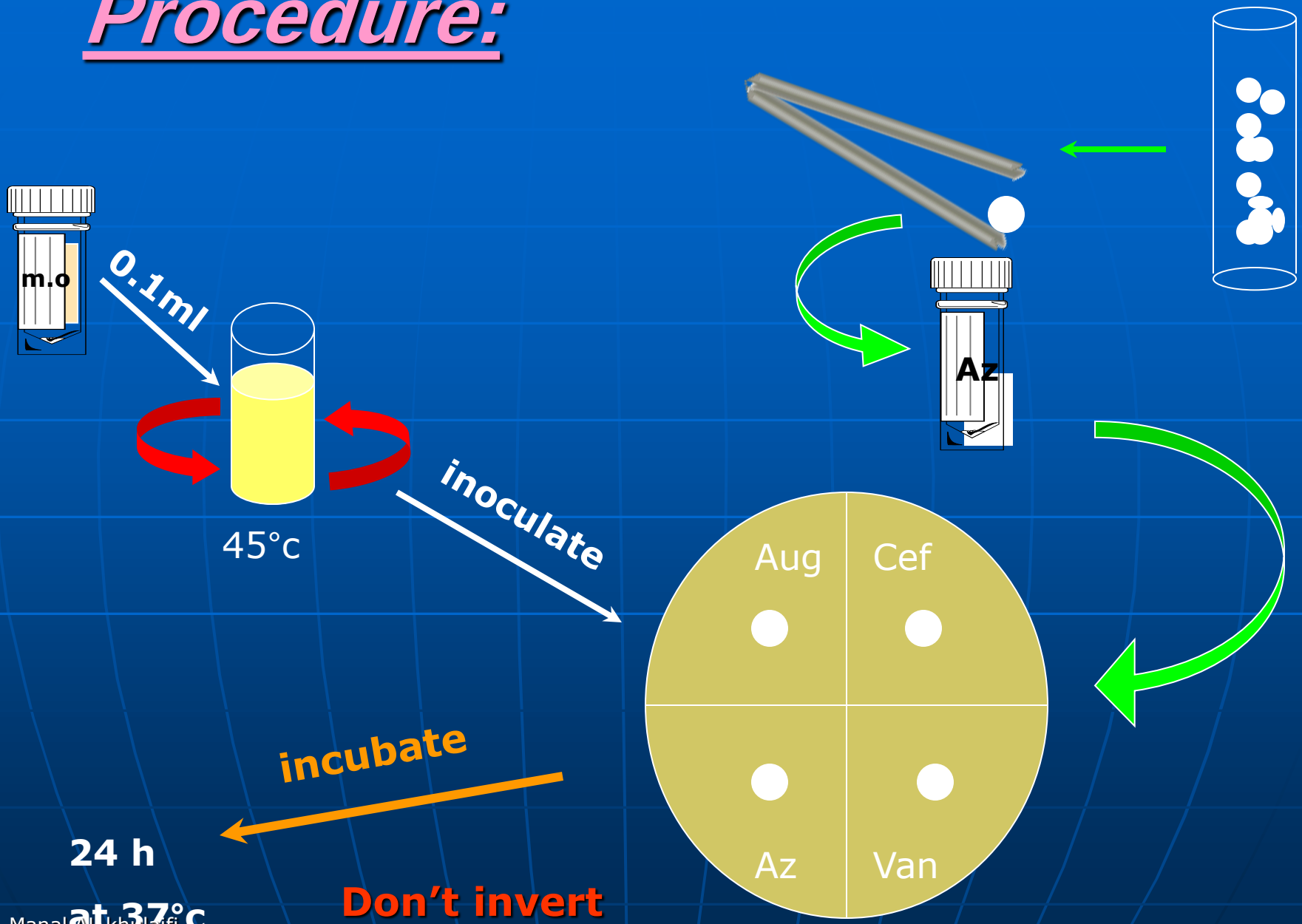
Standardize inoculum suspension

Swab plate

Add disks

Incubate overnight

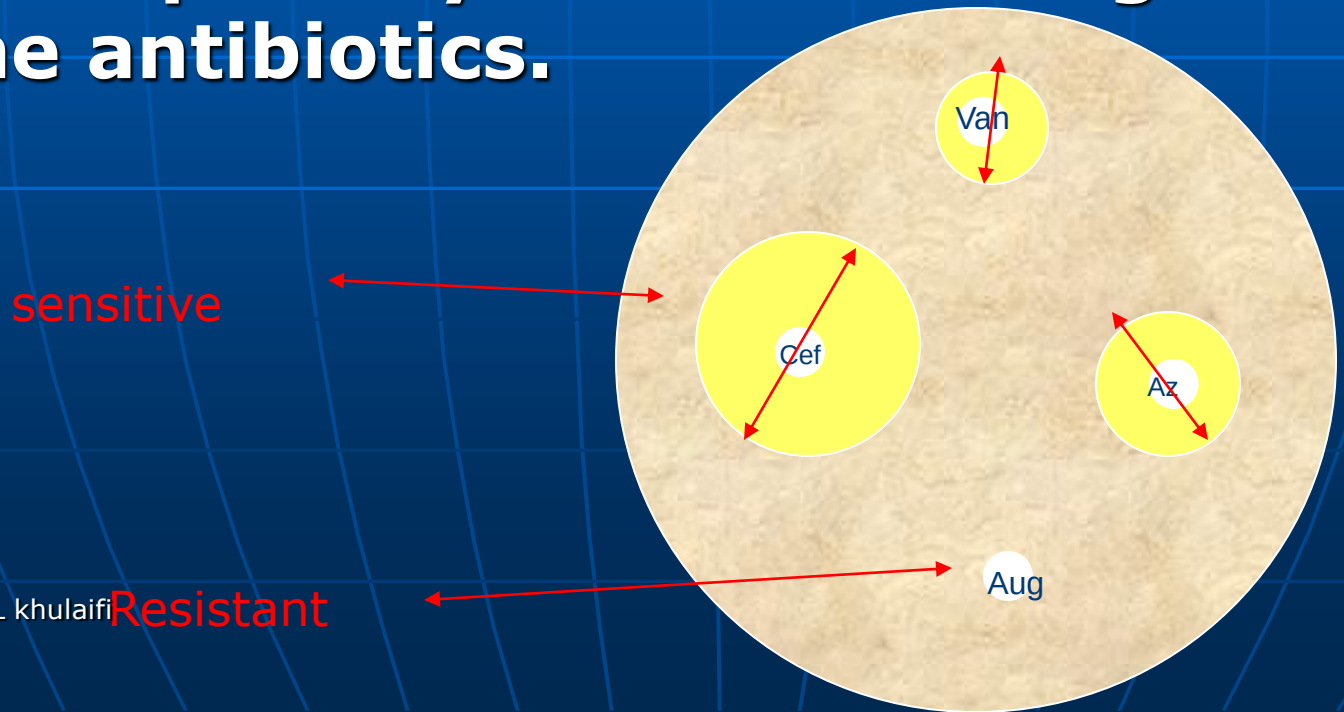
Procedure:


m.o
0.1ml
45°c
inoculate
Az
Aug
Cef
Az
Van
incubate
24 h
at 37°c
Don't invert

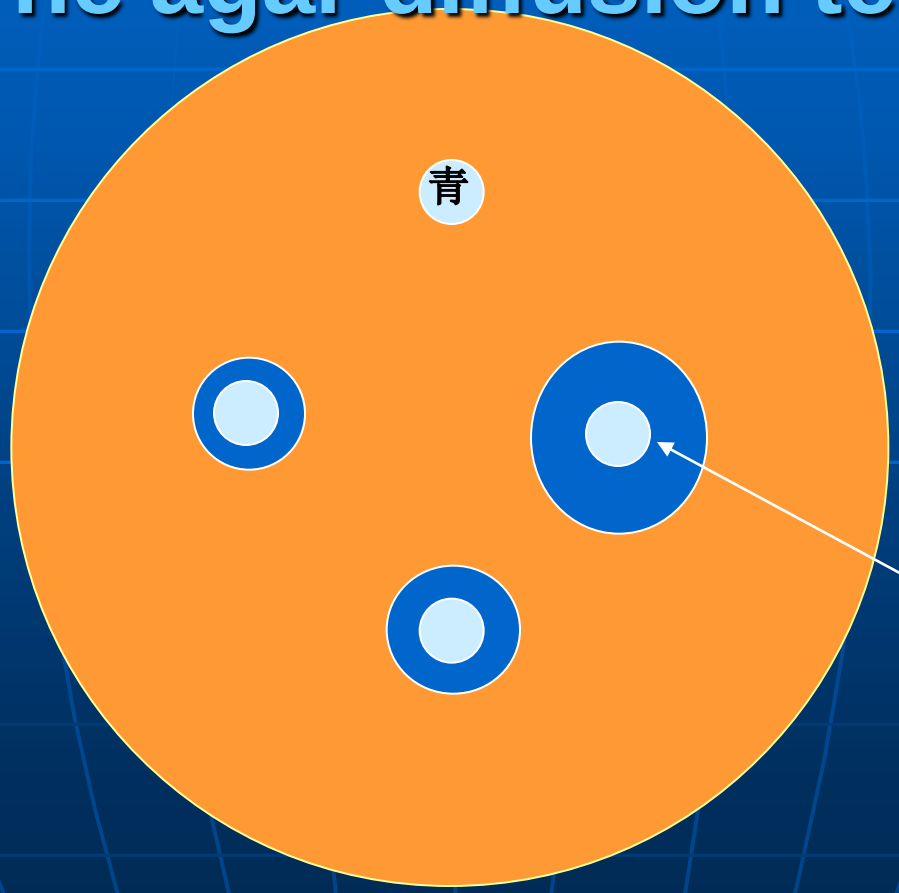
Results:

- Measure the diameter of each inhibition zone

* The diameter of the inhibition zones are directly proportional to the susceptibility of the microorganism to the antibiotics.



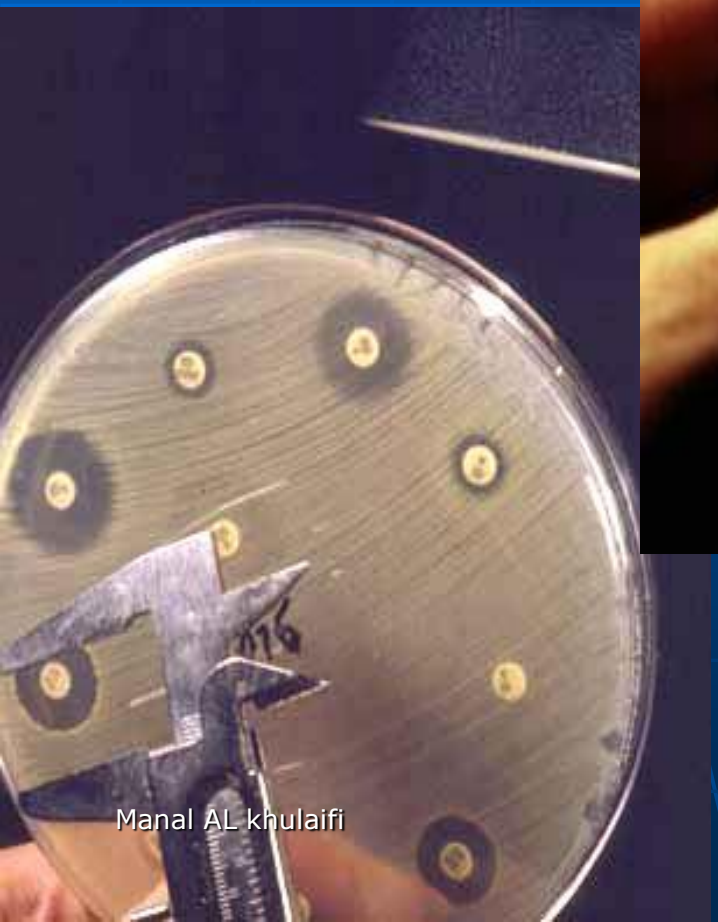
Microbial Antibiotic Susceptibility Test
（The agar diffusion test ）

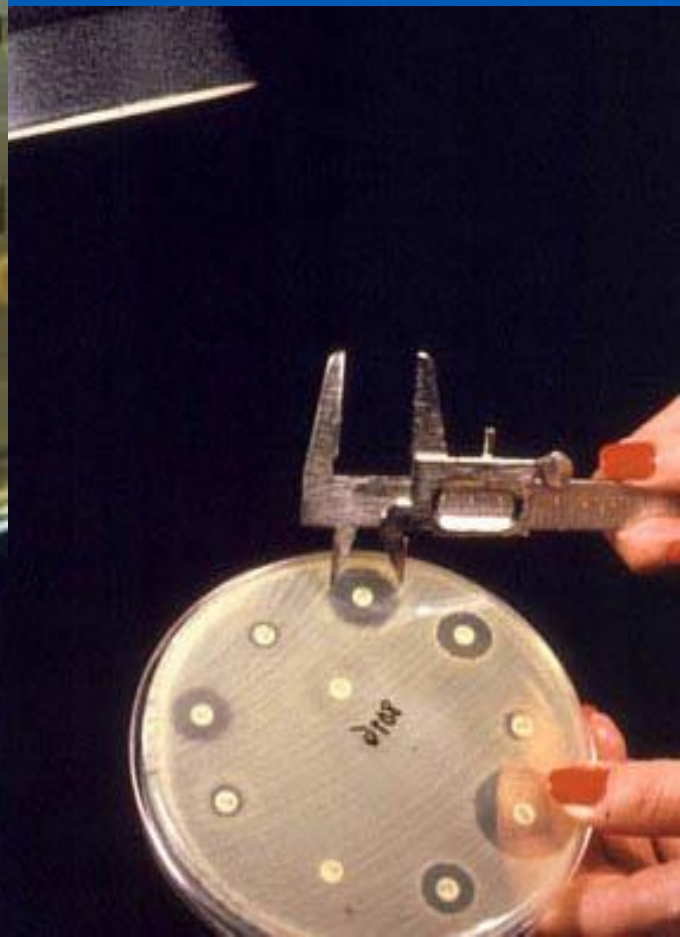


Measure Zones

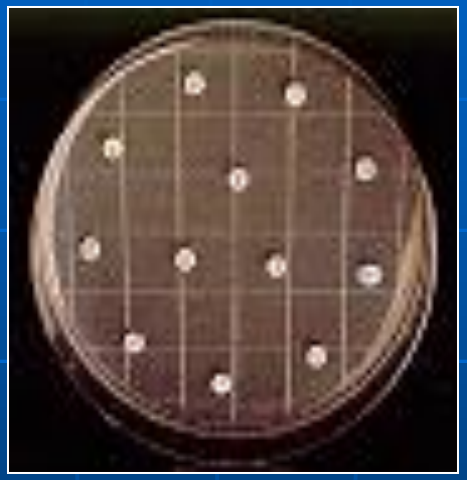
Transmitted Light

Reflected Light





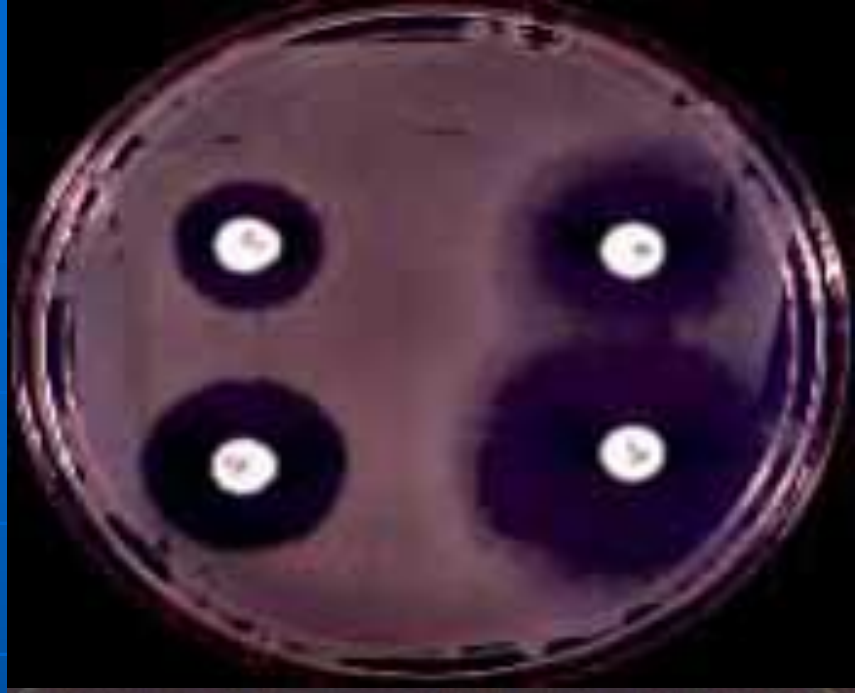
Susceptibility Testing Methods

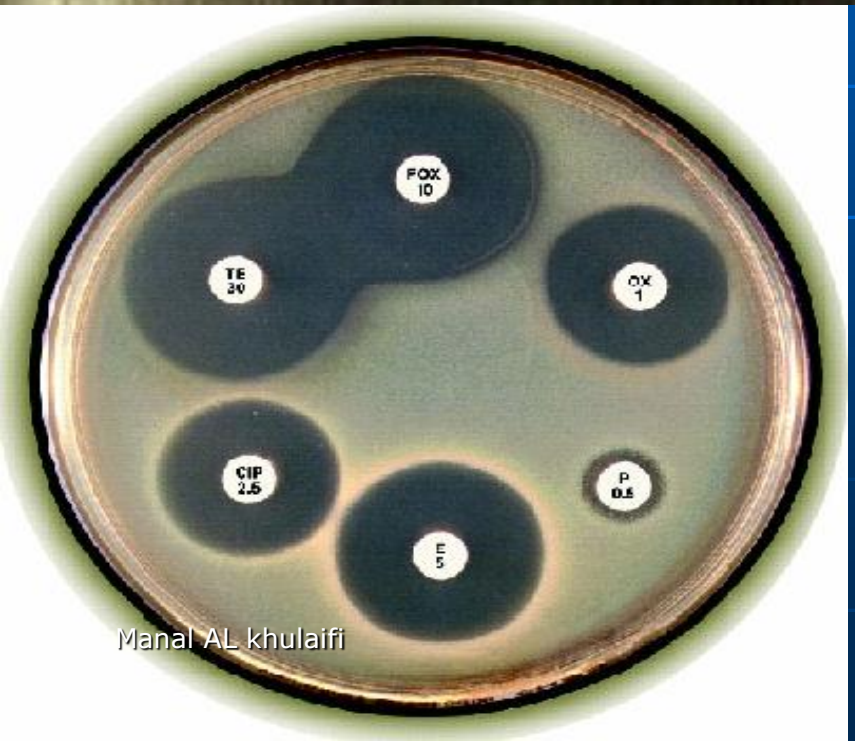


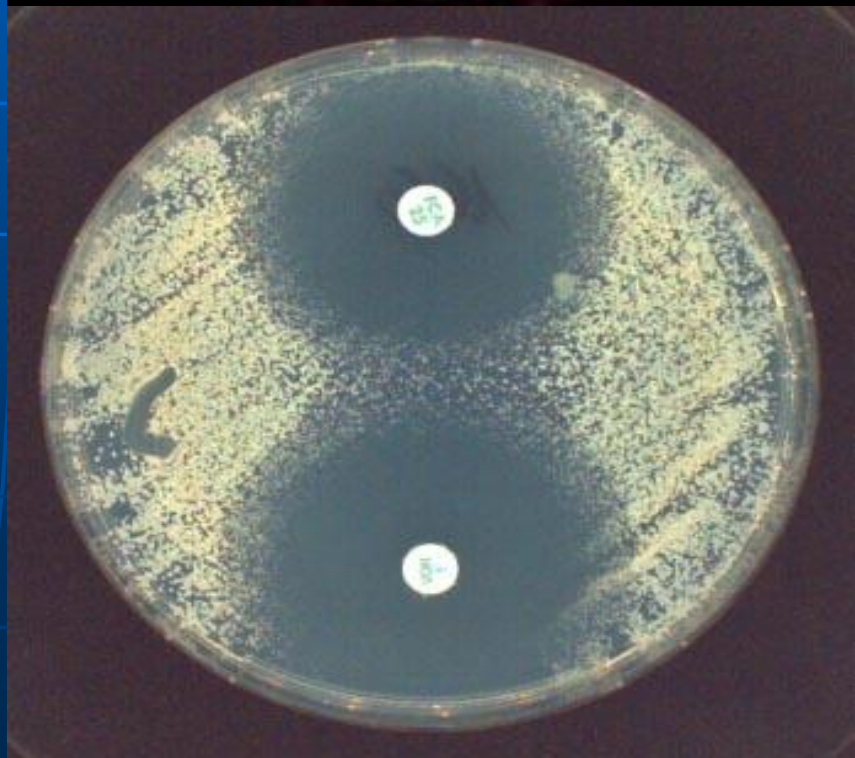
Innoculate MH plate

Place disks on agar plate

Incubate plate 18-24 hr, 35 C Measure and record zone of inhibition around each disk







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Disk Diffusion Susceptibility Testing

MIC:

It is the **lowest concentration of the antimicrobial agent that inhibits the growth of the test organism** but not necessarily kills it.

MBC (minimum bactericidal conc.):

It is the **lowest concentration of the antimicrobial agent that kills the test organism.**

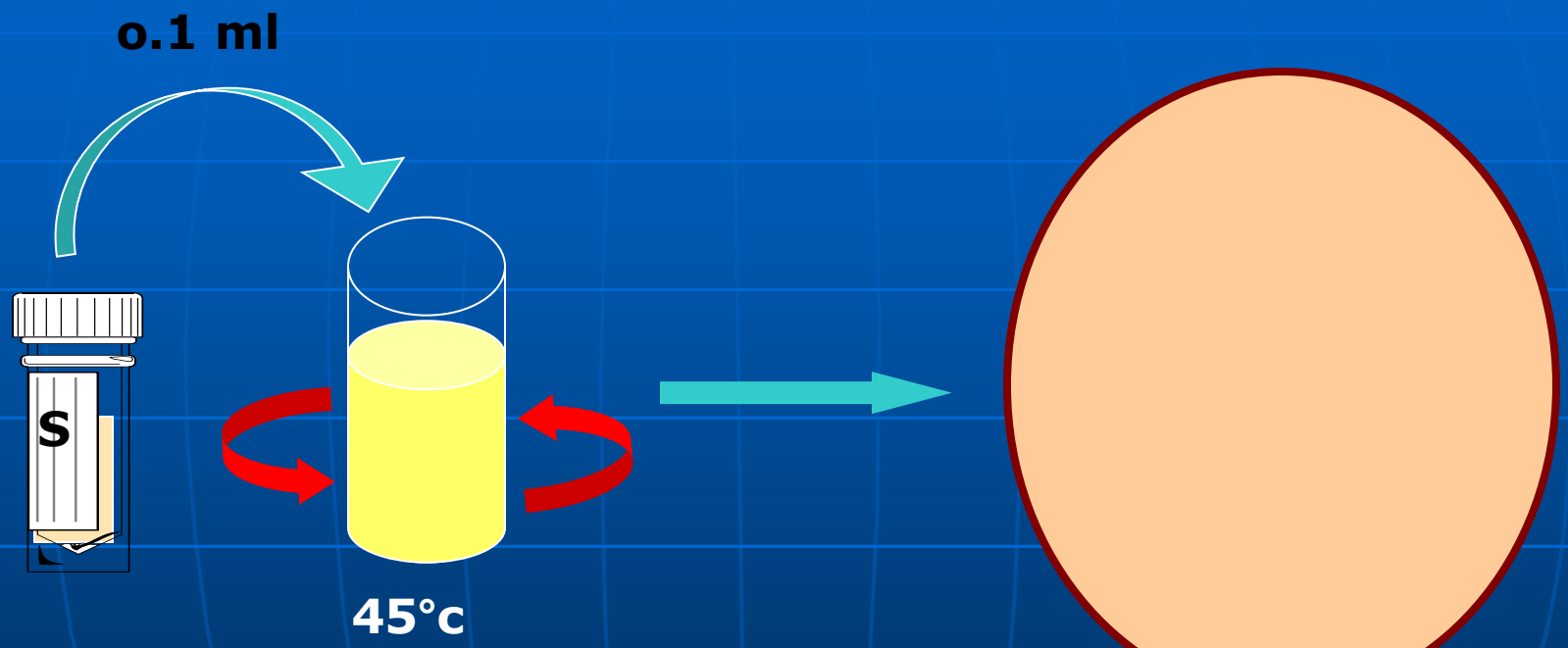
Clinical Conditions when MICs are Useful

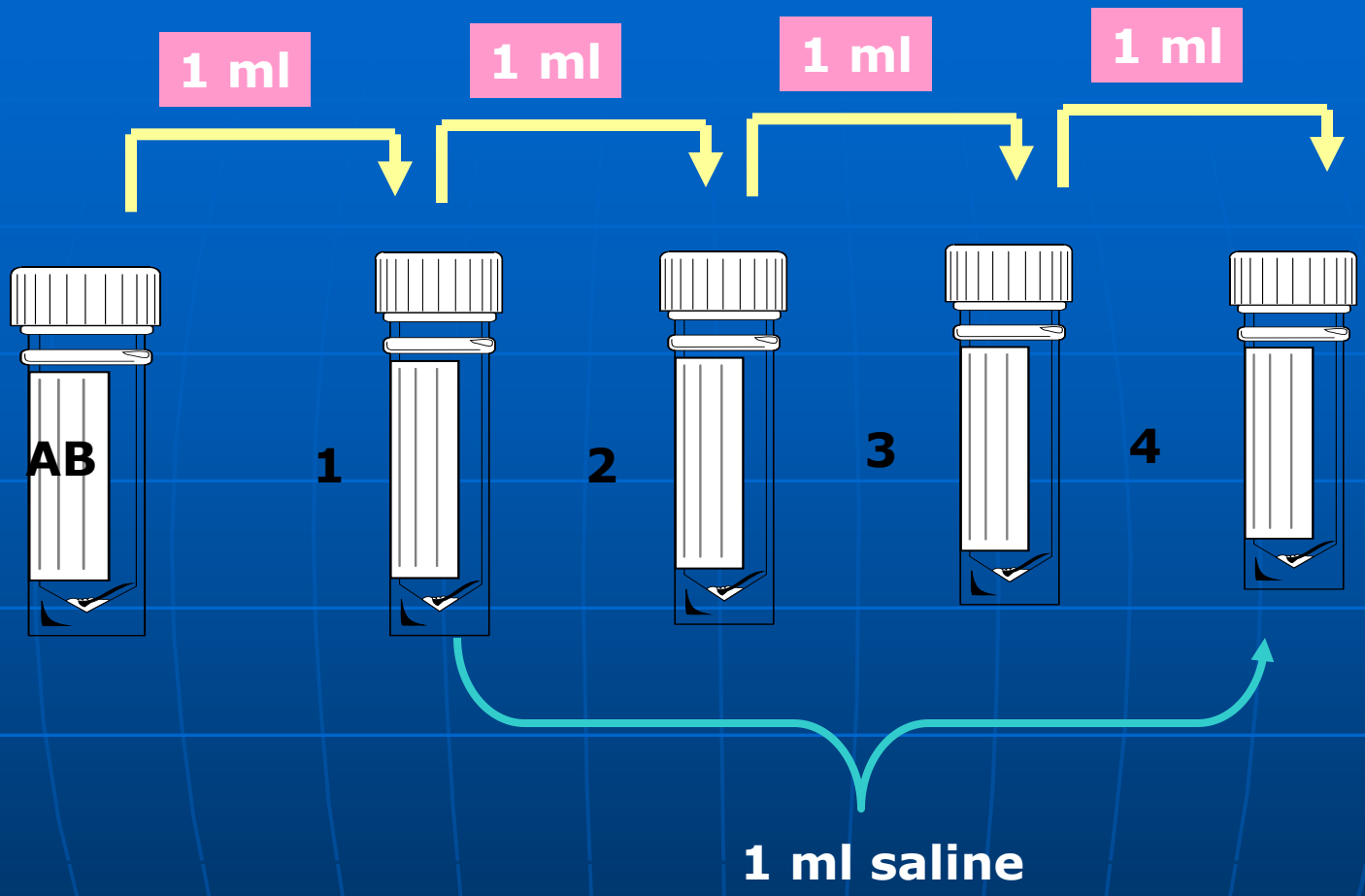
- Endocarditis
- Meningitis
- Septicemia
- Osteomyelitis
- Immuno suppressed patients (HIV, cancer, etc.)

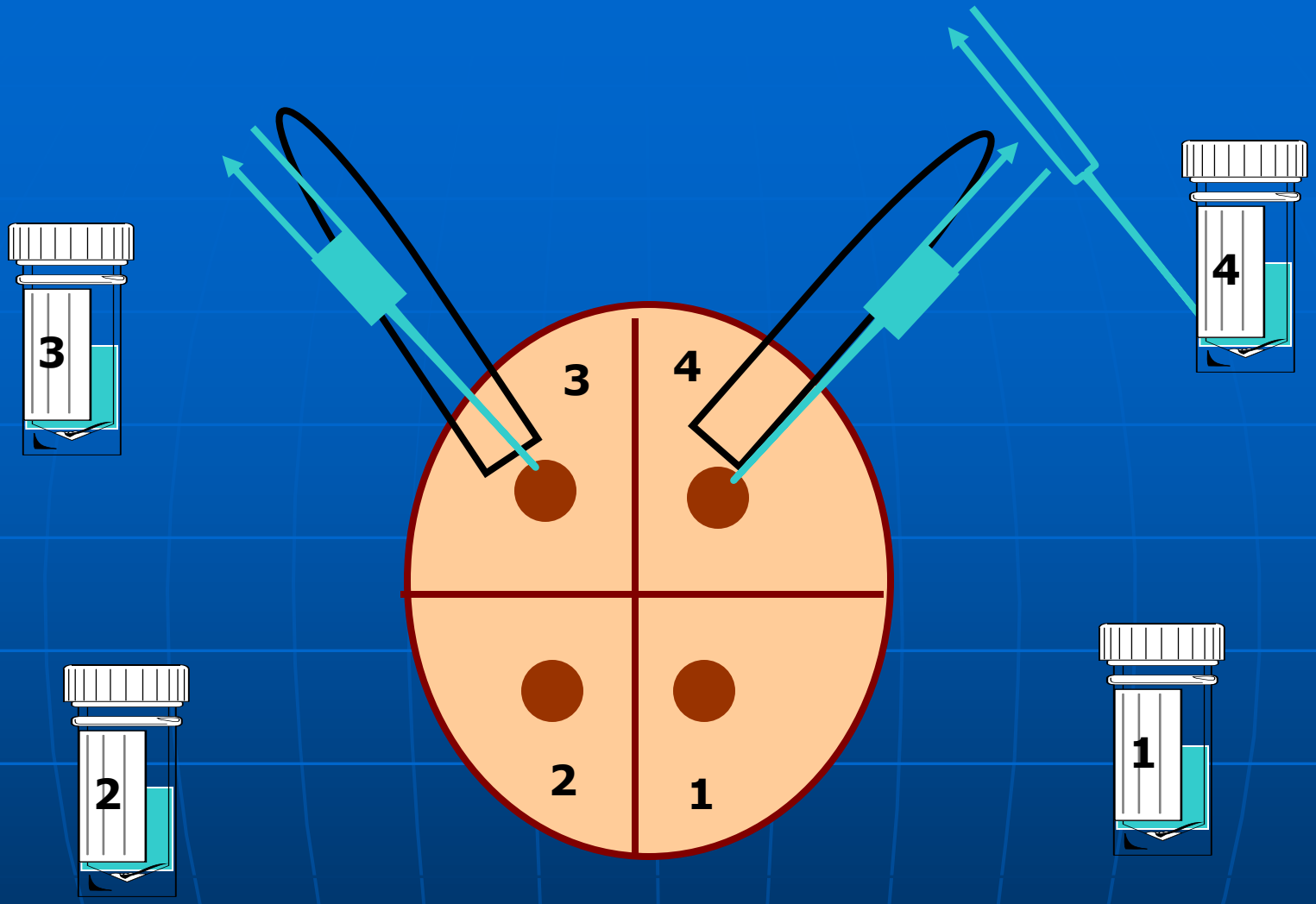
Methods for determination of MIC

1. **Serial dilution** method
2. **Agar diffusion** method
3. **E- test**

Procedure:




1 ml
1 ml
1 ml
1 ml
AB
1
2
3
4
1 ml saline



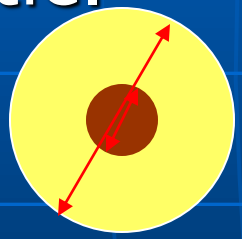
Incubate the plate Uninverted at 37°c for 24h

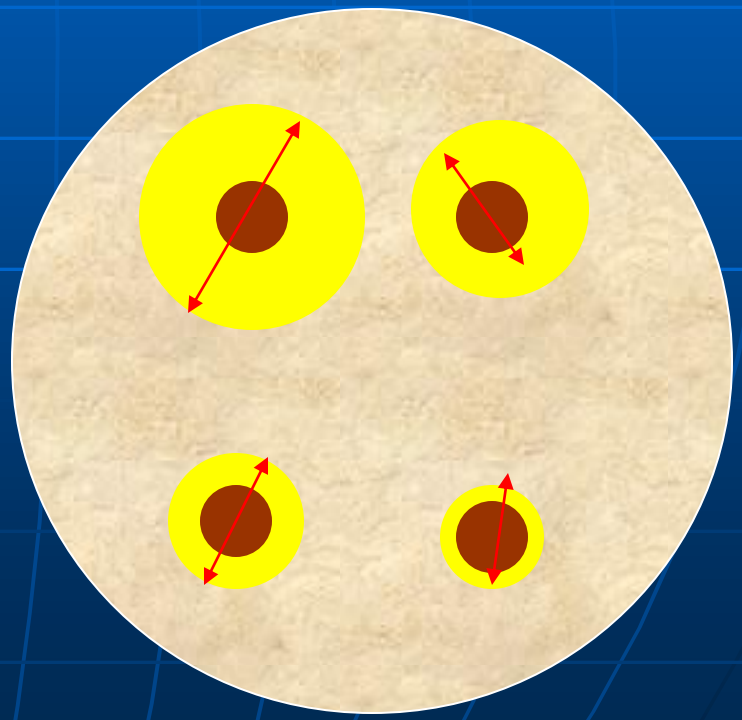
Results:

- Measure the diameter of each inhibition zone
* The diameter of the inhibition zones are directly proportional to the conc. of the antibiotic.

Zone diameter

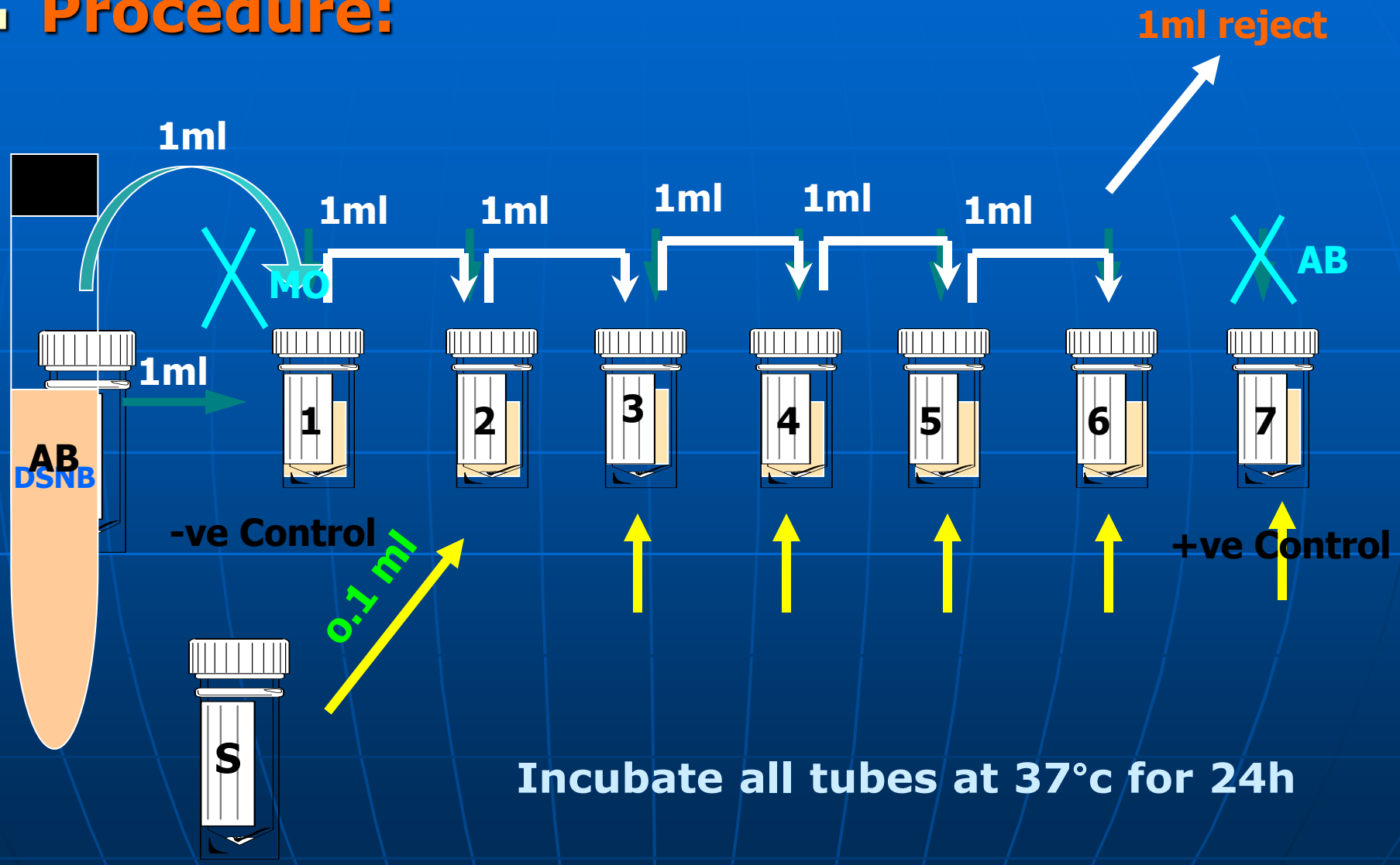
Well diameter



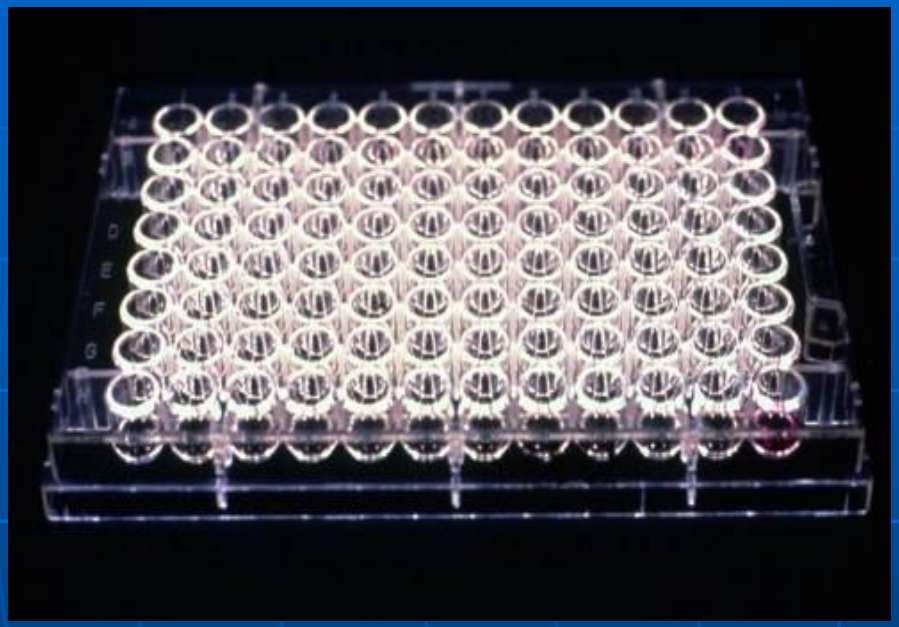


Determination of MIC by the serial dilution method

- **Procedure:**



Prepare inoculum suspension



Microdilution MIC tray

F-test

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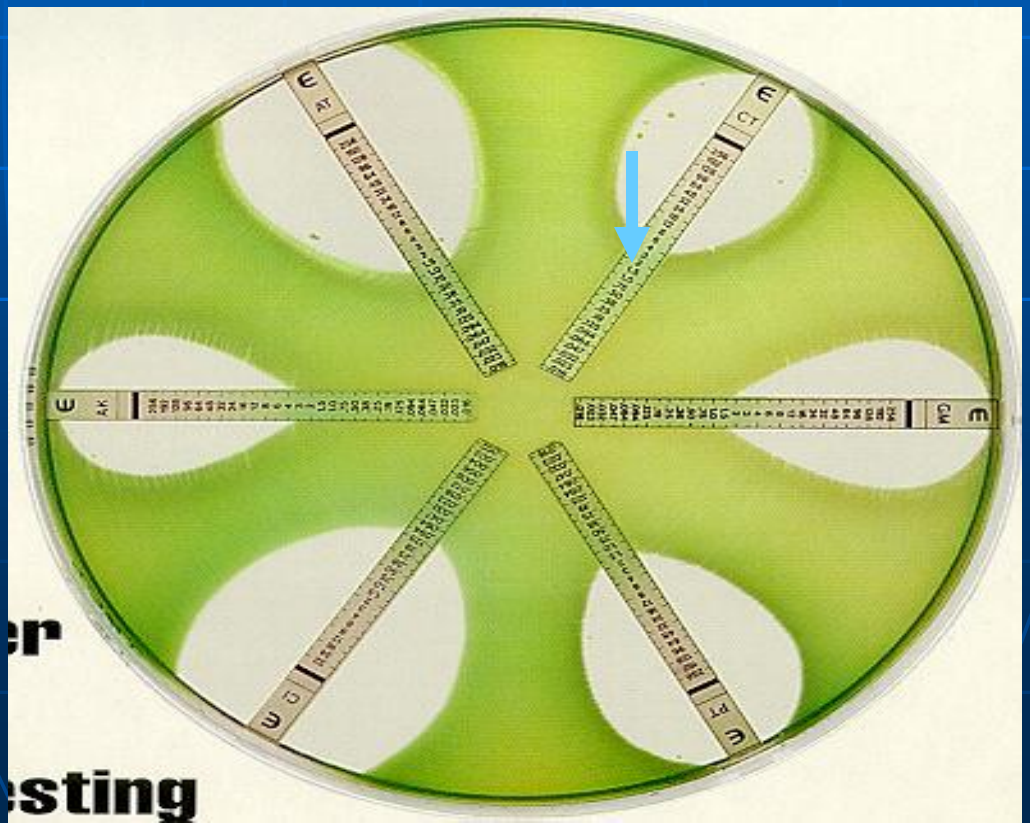
Antimicrobial Gradient Testing
E-test®

A plastic coated strip contains a gradient of antibiotic concentrations and the minimal inhibitory concentration is read from a scale printed on the strip

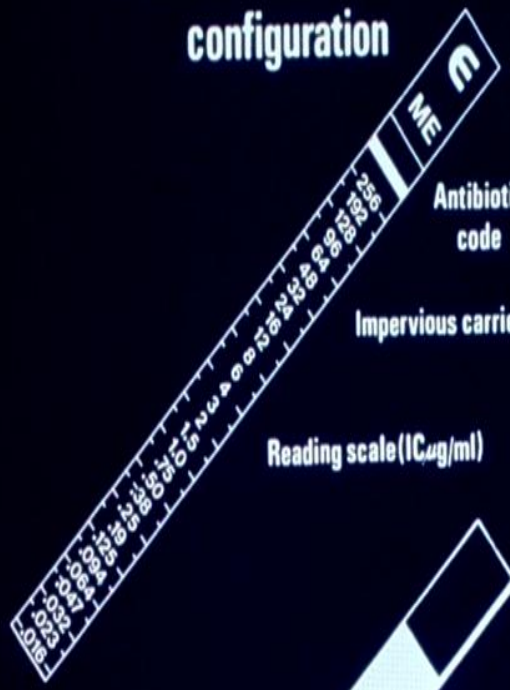
Read plates after recommended Incubation

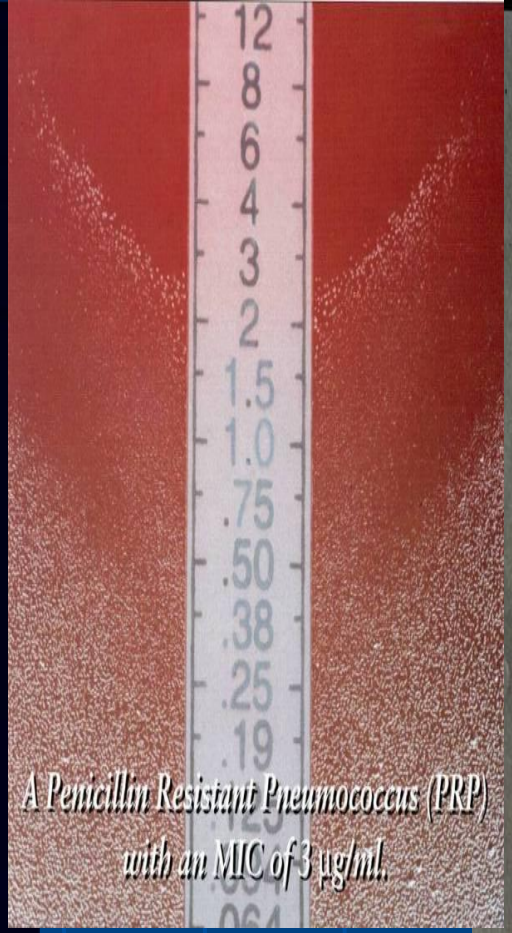


Read MIC where intersects scale

the E test

configuration





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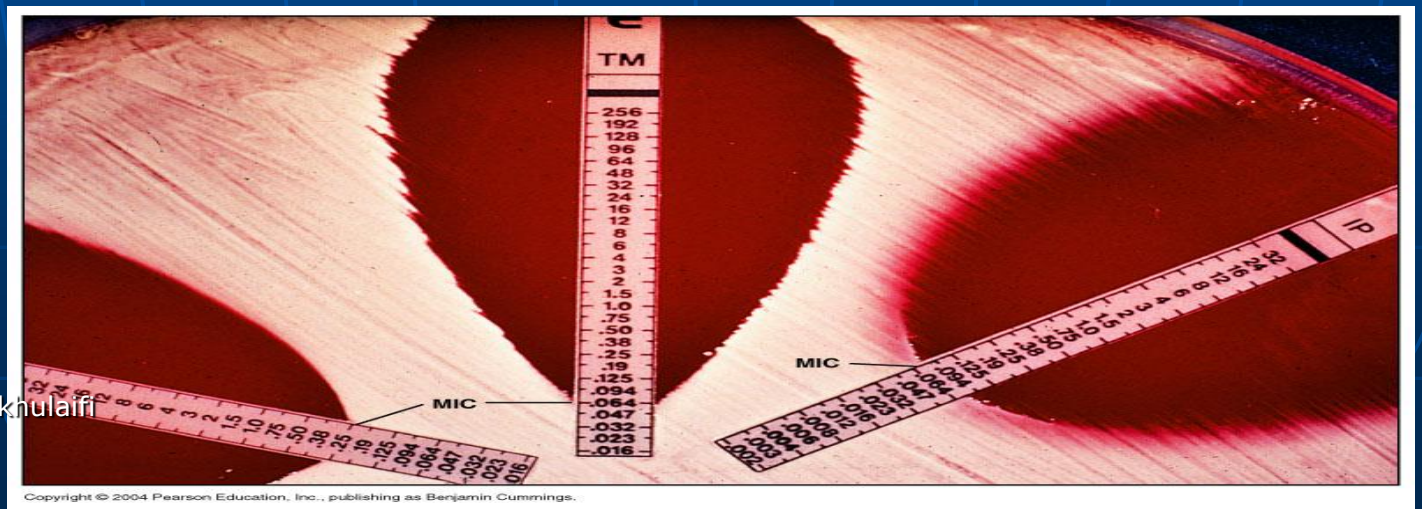
E-Test



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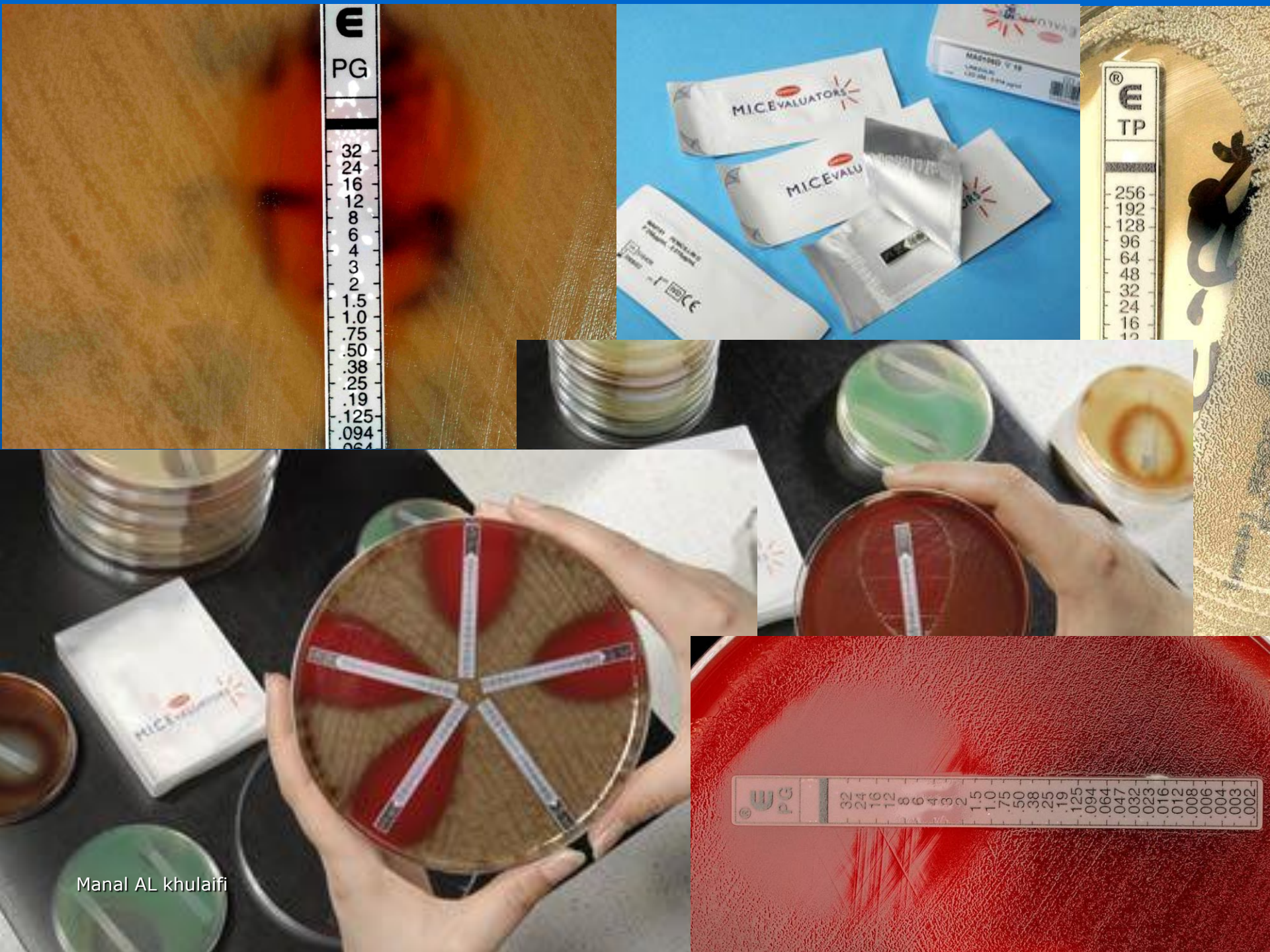
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E-test fer *P.aeruginosa*