BHARATHIDASAN UNIVERSITY

Tiruchirappalli-620024
Tamil Nadu, India.

Programme: M.Sc., Biomedical Science

Course Title : Microbiology

Course Code : BM24AC4

Unit-II

Antibiotic Sensitivity Test

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ANTIBIOTIC SENSITIVITY TEST PROCEDURE AND ITS QUALITY CONTROL

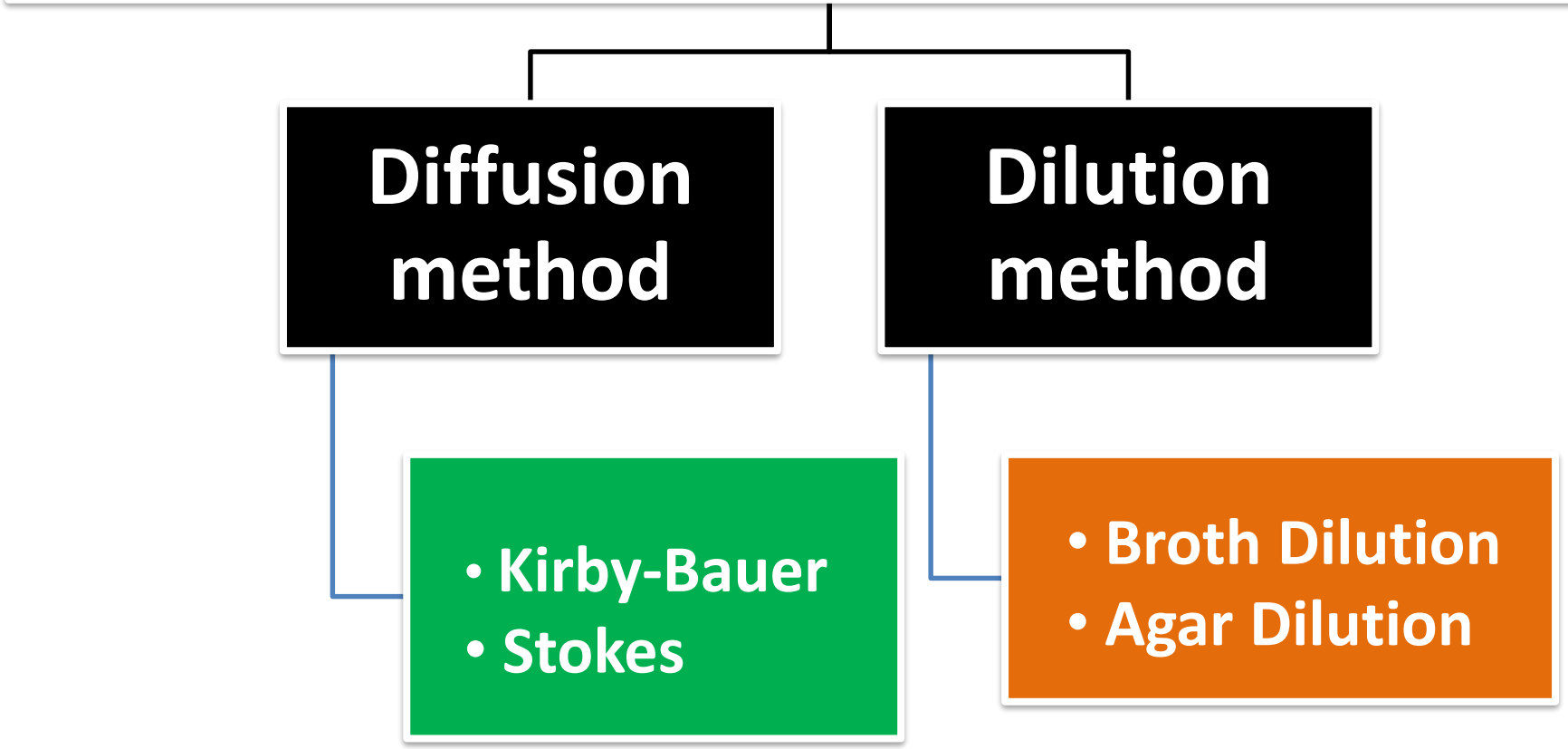
Bacteria exhibit great strain variations in susceptibility to antimicrobial agents

Essential to determine the susceptibiliiy of pathogenic bacteria isolated from the clinical specimens to antibiotics

Antimicrobial Susceptibility Testing (AST)

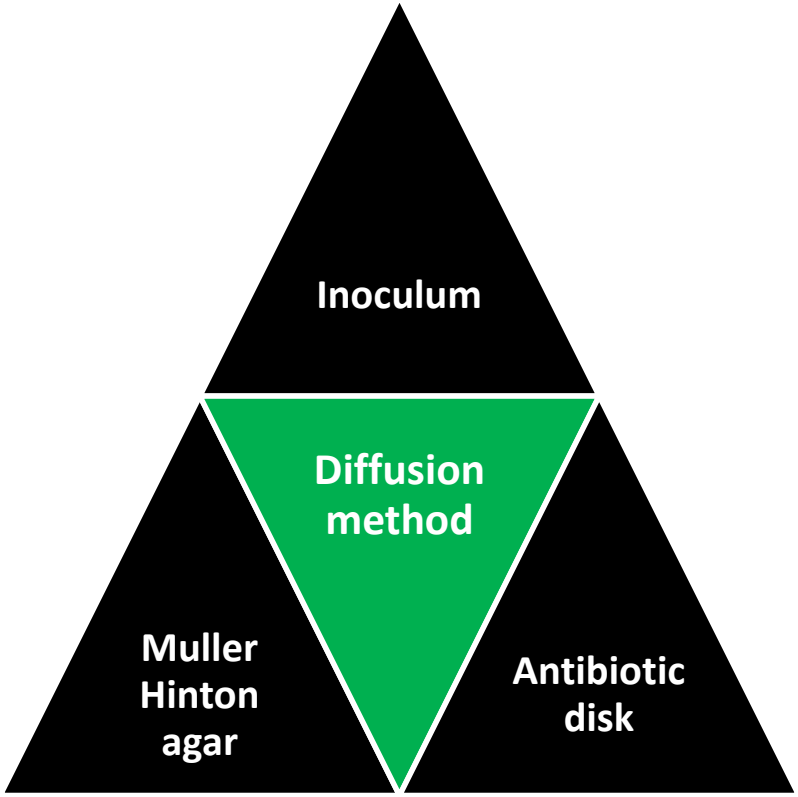
AST is performed only for pathogenic bacteria isolated from the specimen and not for the commensal bacteria

Antimicrobial Susceptibility Testing



Disk Diffusion Method

- **Widely used method**
- **Suitable for rapidly growing bacteria (eg- *Staphylococcus,Pseudomonas*)**



Non-differential and Non-selective medium

Shows acceptable batch-to-batch reproducibility for susceptibility testing

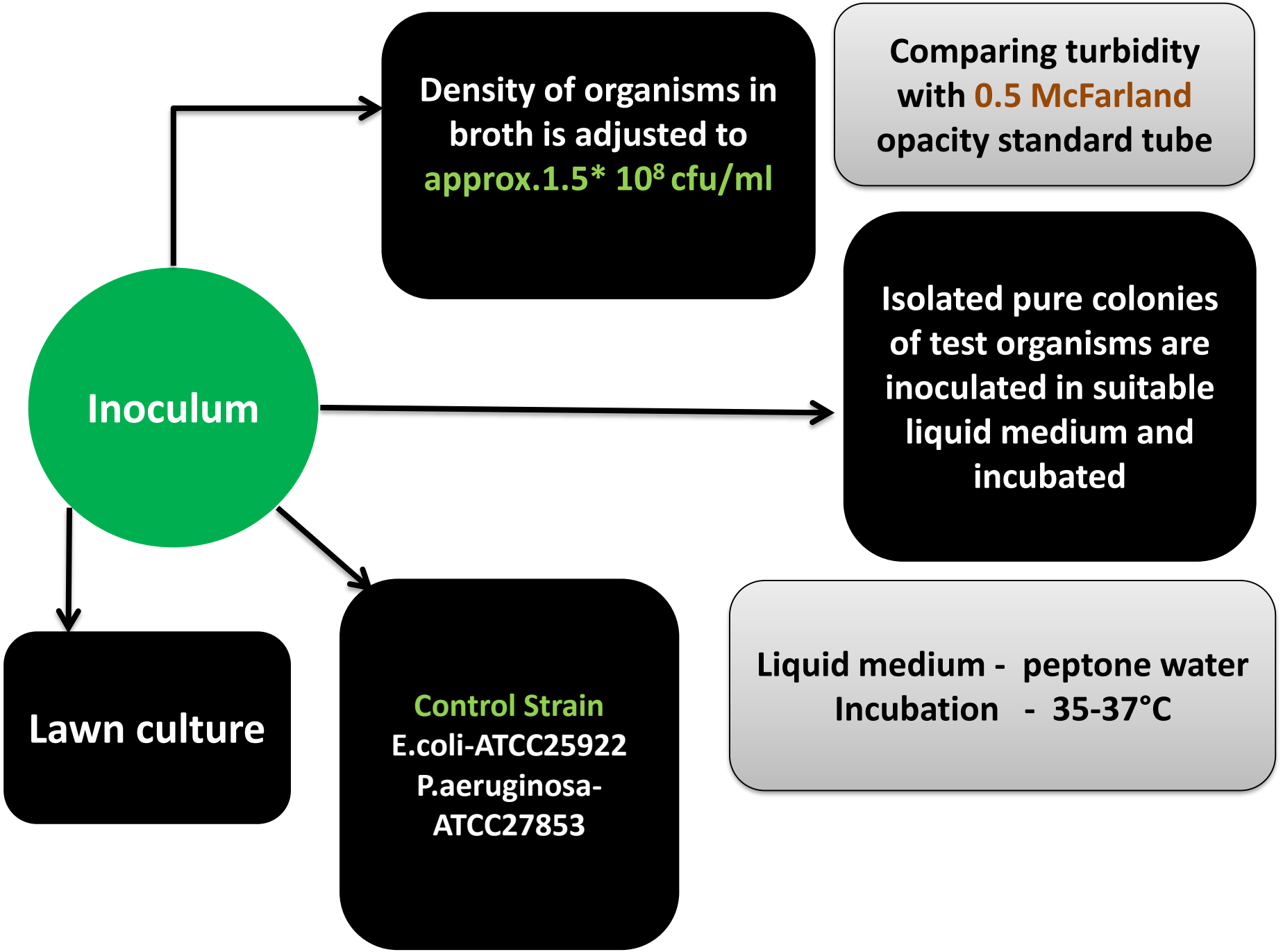
Muller Hinton Agar

ph-7.2 -7.4

Satisfactory growth medium for most non-fastidious bacteria

Minimal inhibitory effect on Sulfonamide and Trimethoprim

- **Lysed horse + MHA = supports fastidious organism (*H.influenza*)**
- **NaCl (2-4%) + MHA = MRSA (Methicillin resistant *Stahphylococcus aureus)***


Density of organisms in broth is adjusted to approx.1.5* 10^8 cfu/ml
Comparing turbidity with 0.5 McFarland opacity standard tube
Inoculum
Isolated pure colonies of test organisms are inoculated in suitable liquid medium and incubated
Lawn culture
Control Strain
E.coli-ATCC25922
P.aeruginosa-ATCC27853
Liquid medium - peptone water
Incubation - 35-37°C

Whatman no : 1

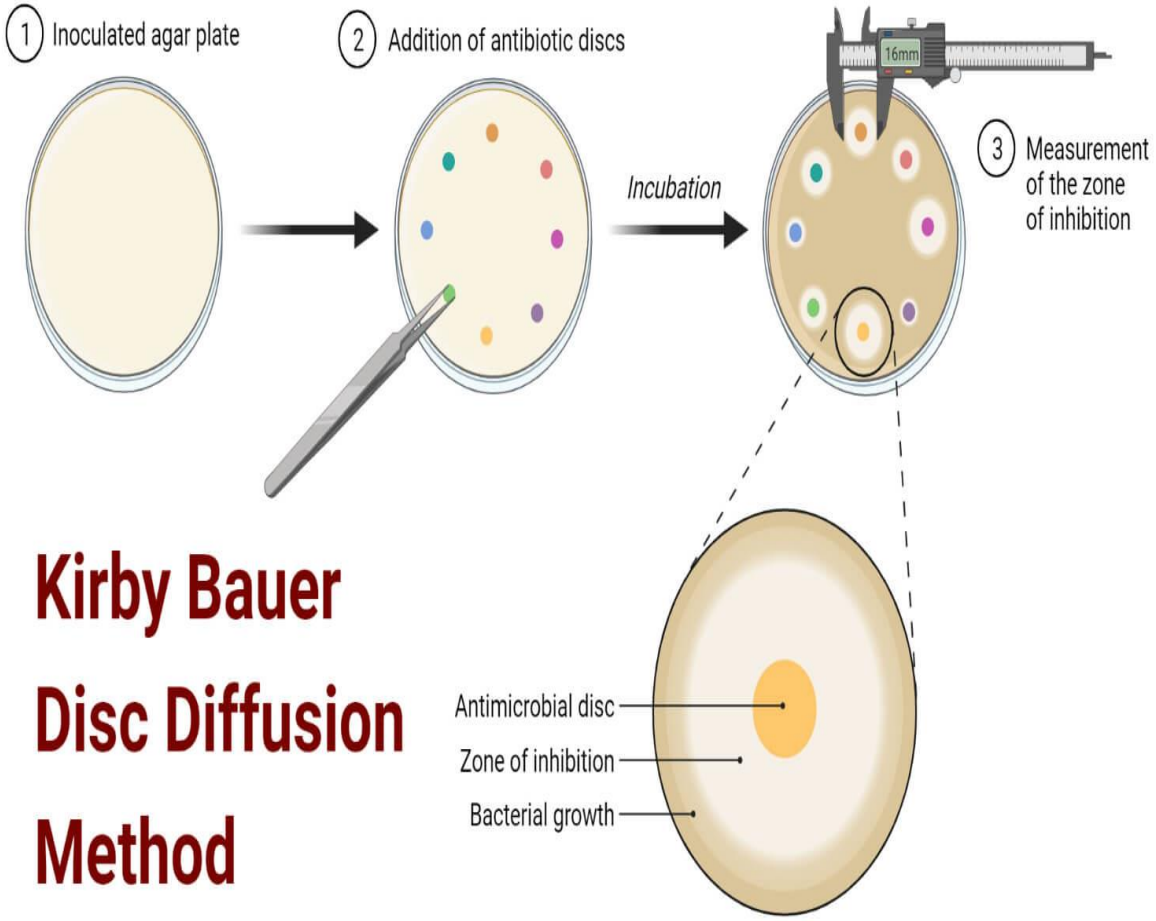
Diameter - 6mm

Bulk stock stored at -20°C and working stock at 8°C

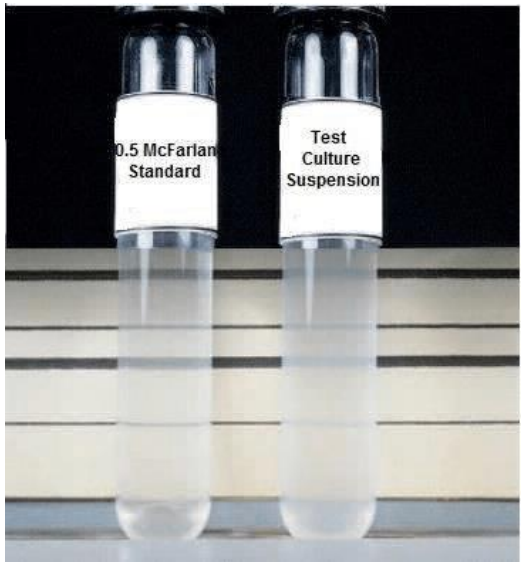
Antibiotic disk

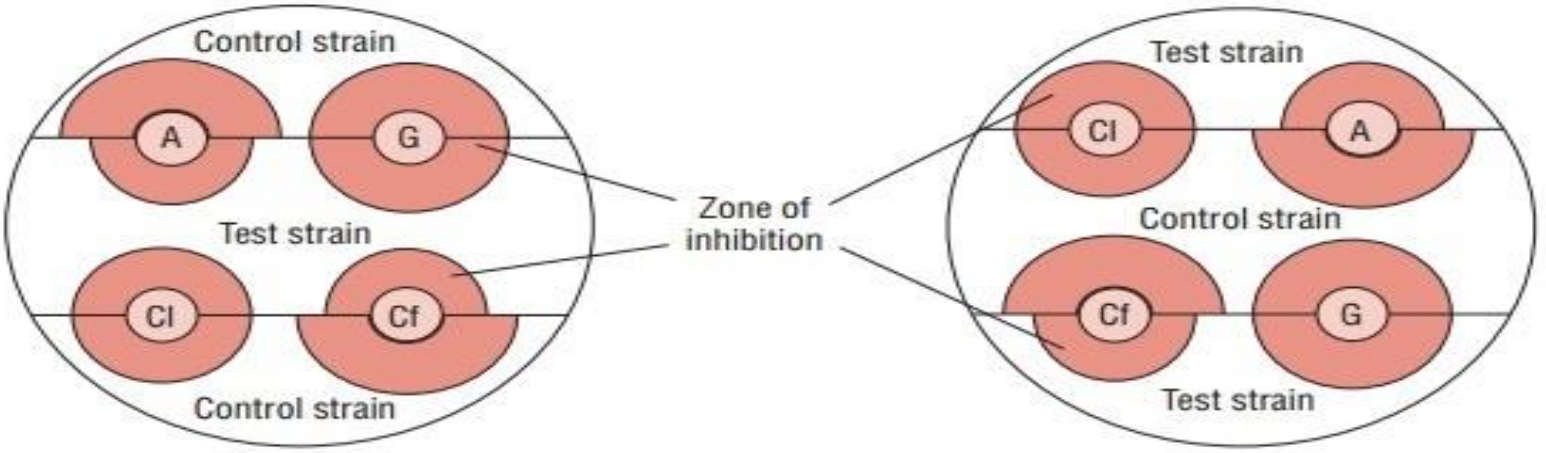
Antibiotic delivered with 20 gauge wire loop

Should be stored in sealed containers with dessicants

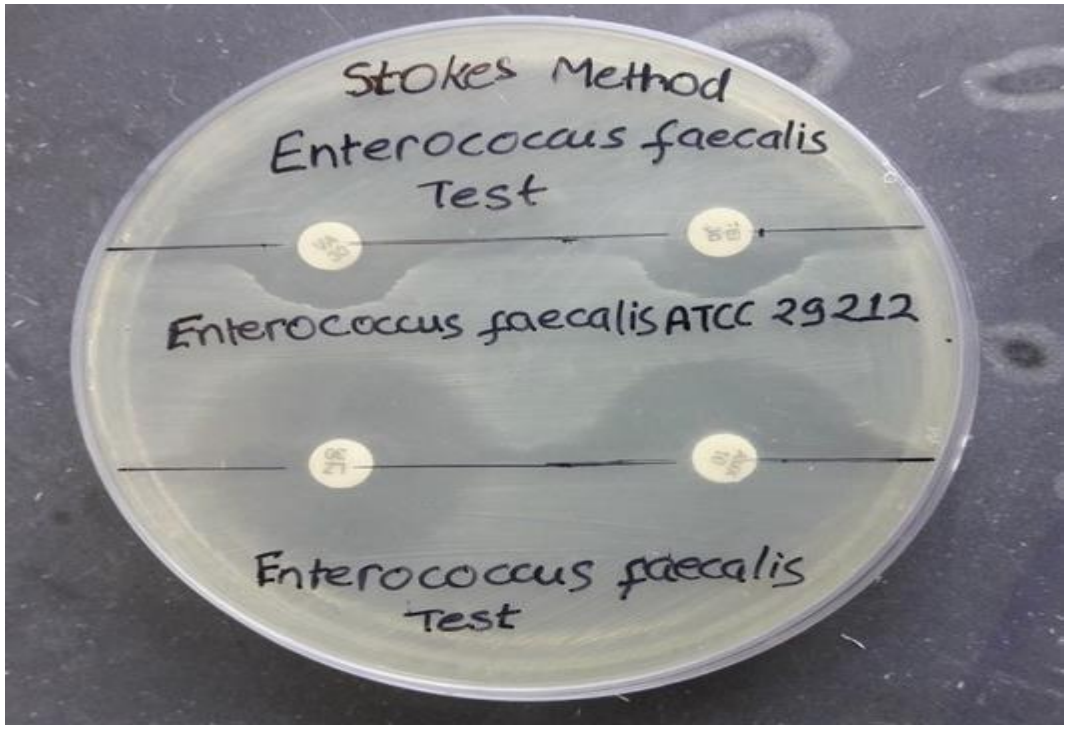


Kirby Bauer Disc Diffusion Method



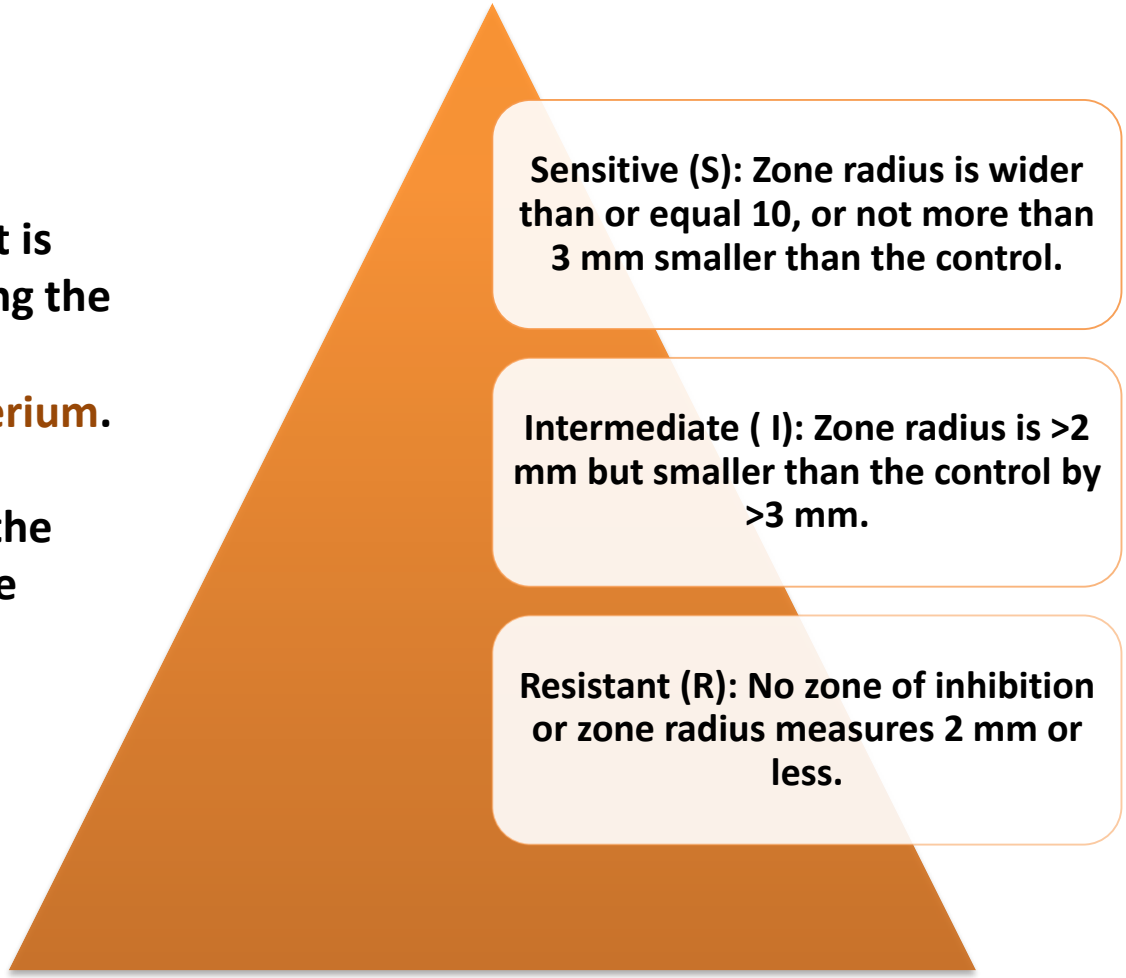


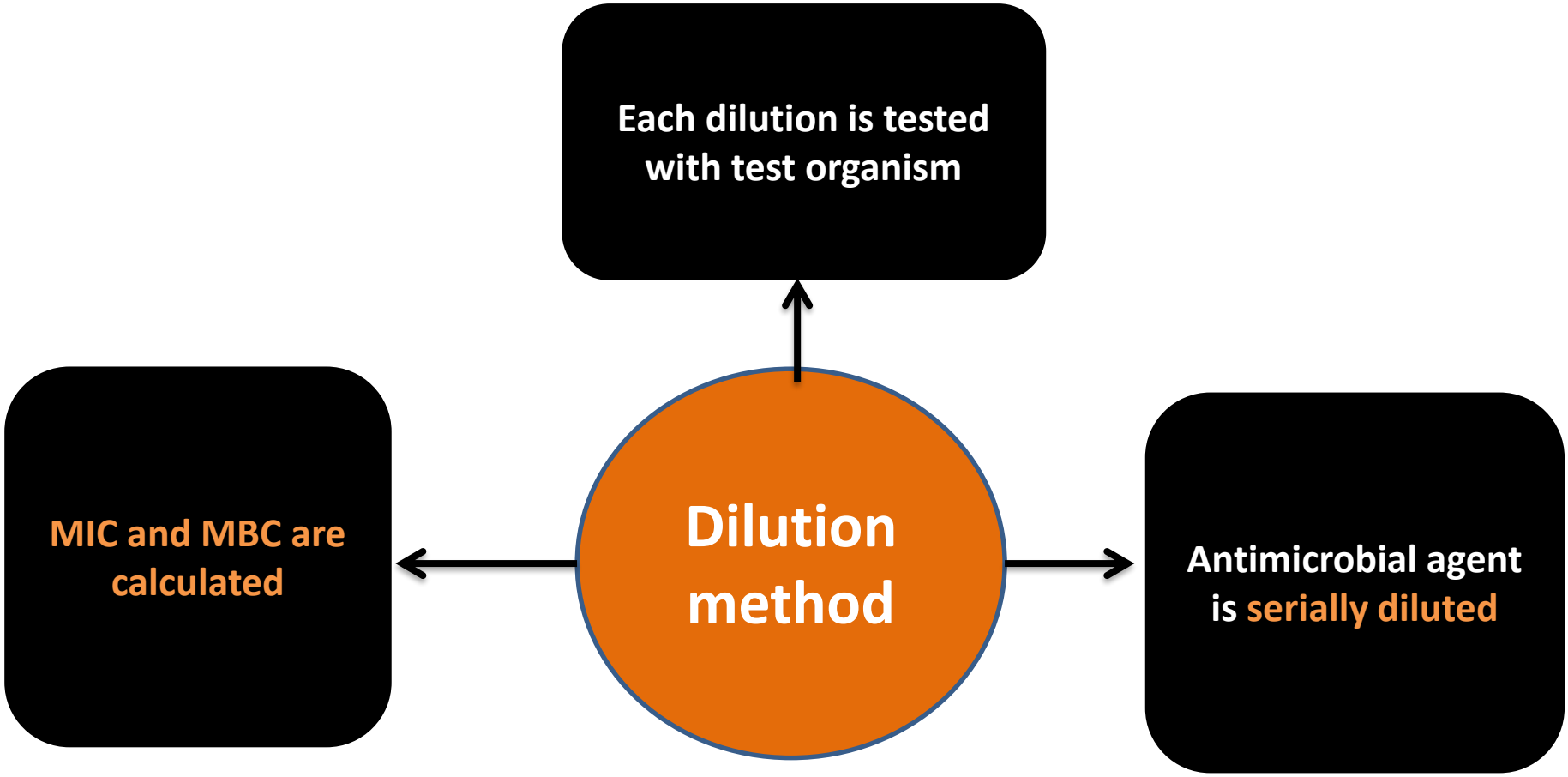
Schematic diagram showing Stokes method of antibiotic sensitivity.



Reporting in Stokes Method

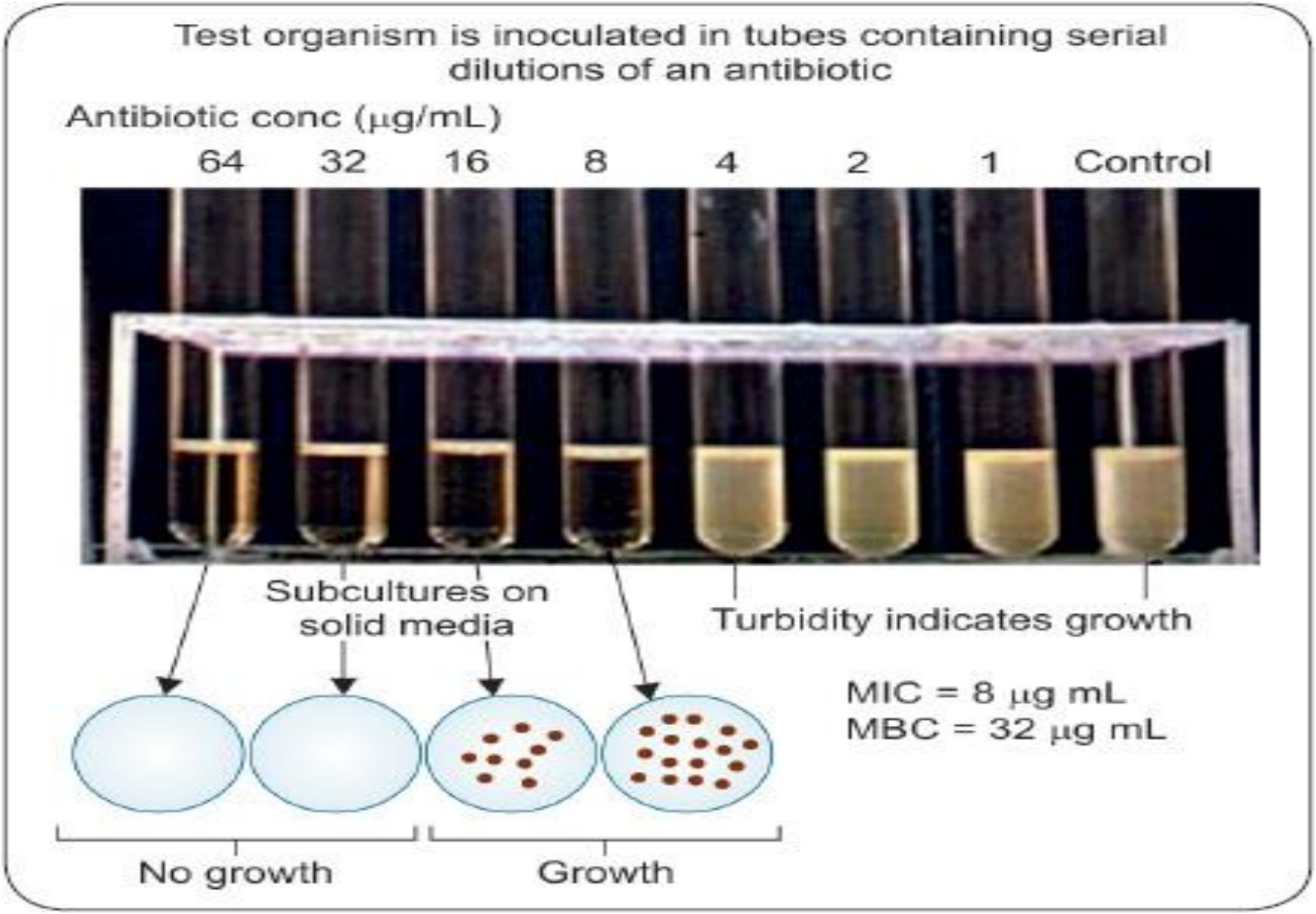
- The sensitivity report is prepared by comparing the zones of inhibition of control and test bacterium.
- The radius of the inhibition zone from the edge of the disk to the edge of the zone is measured.



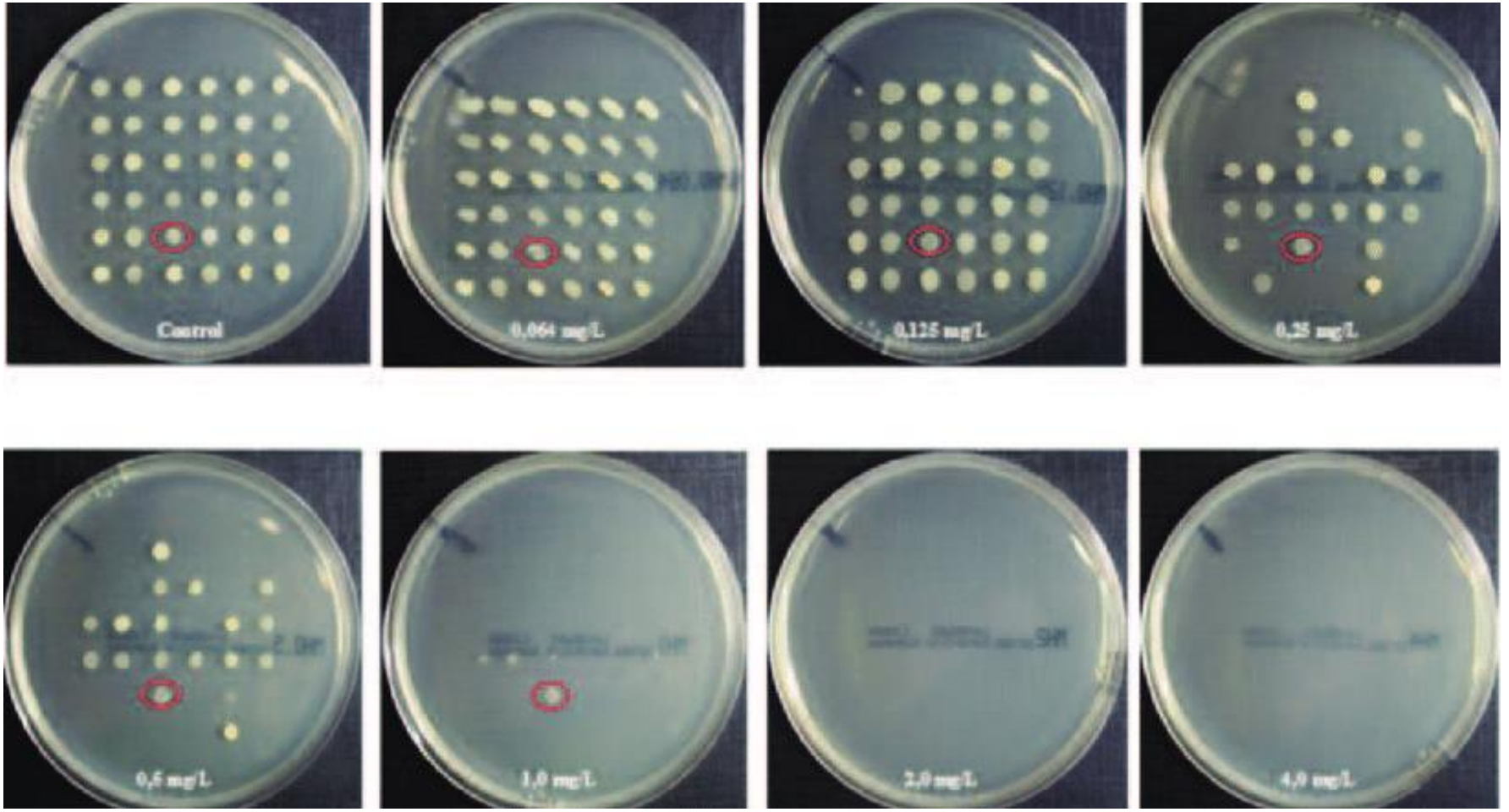


- **MIC** - the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation
- **MBC** - the minimum concentration of drug which kills 99.9% of the test microorganisms in the original inoculum.

Broth Dilution method

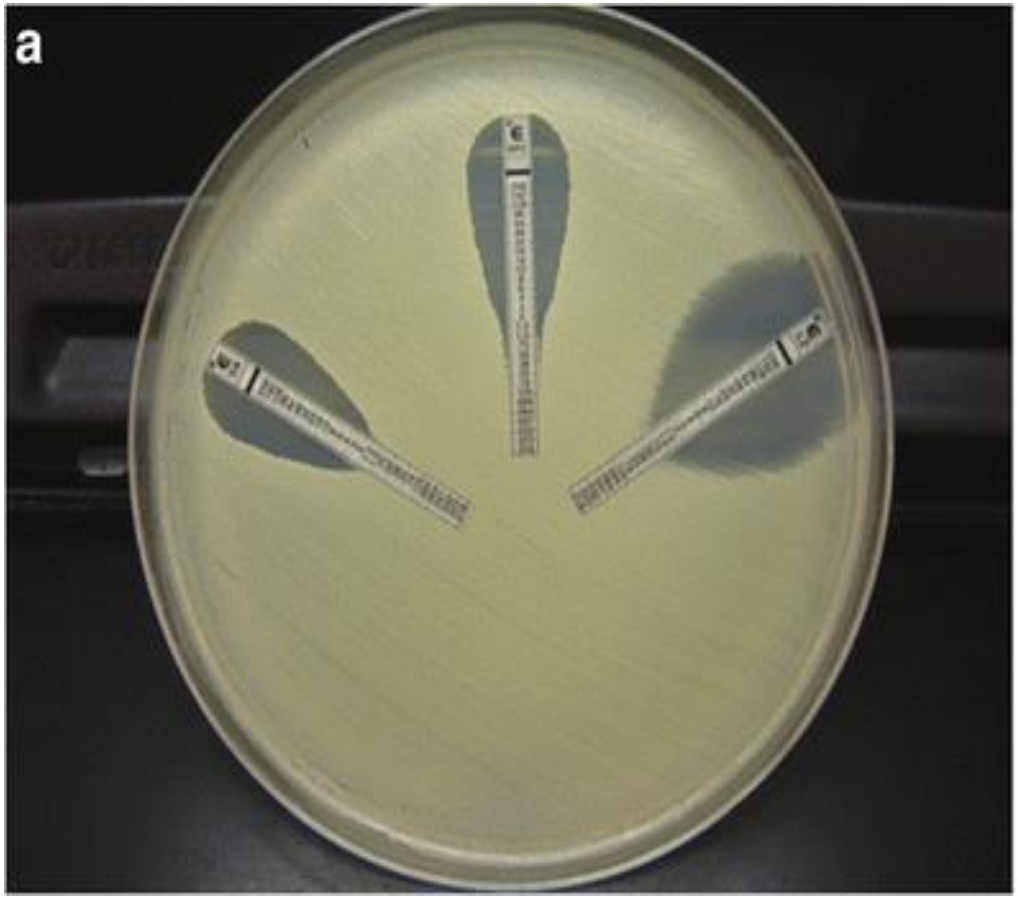


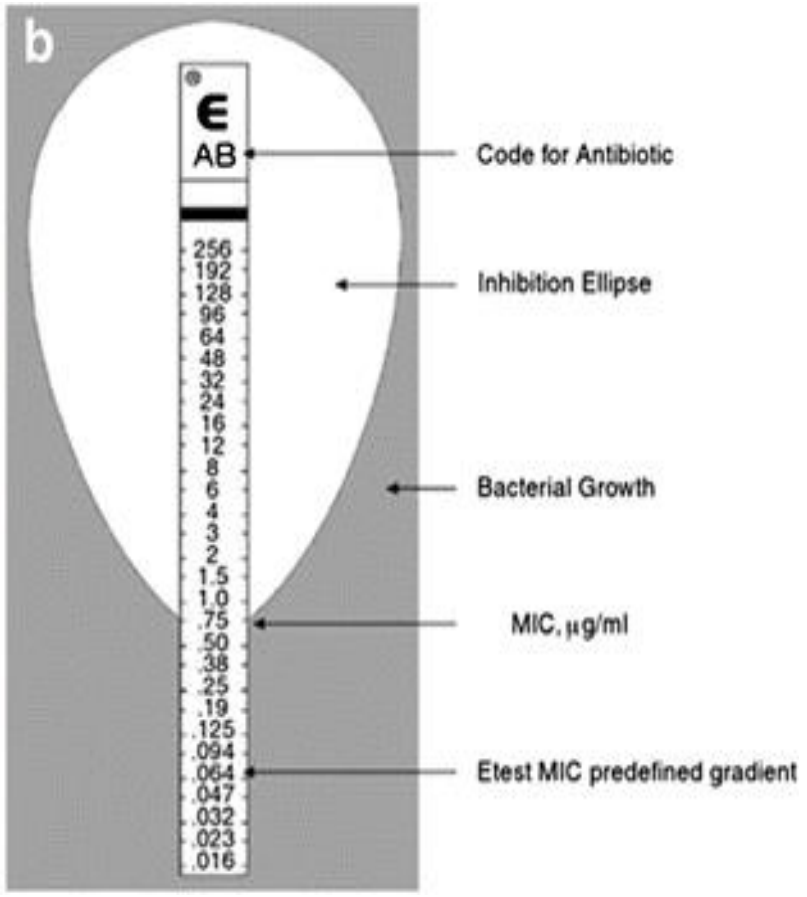
Agar Dilution Method



Epsilometer Test

- Quantitative test
- using the principles of both dilution and diffusion of antibiotic into the medium.





Quality Control

Goals of quality control is to monitor,

- **Precision (repeatability)**
- **Accuracy of susceptibility test procedures**
- **The performance of reagents used in the test**
- **Performance of persons who carry out the tests and read the results.**

1. **Culture media: Muller-Hinton**
2. **Reagents: disks**
3. **Size of the inoculums**
4. **Incubation condition**
5. **Control with reference strains**
6. **Reading inhibition diameters (accurate measurement)**
7. **Knowledge of staff**

Table 1 : Specifications for performing AST

Medium	Mueller Hinton Agar	4 mm thickness pH : 7.2 to 7.4
Antibiotic discs	Storage temperature	-20 °C minimum
Inoculum	McFarland 0.5	10^8 bacteria/mL
Incubator	Temperature	37° C
	Atmosphere	Ambient air

Table 2 : Factors affecting AST & their effect on it (variables).

Factor	Variables
pH	If pH is low:→ *Aminoglycosides, Quinolones & Macrolides lose potency. * Tetracyclines have excess action.
Moisture	Affects accuracy of susceptibility testing.
Medium component	Excess of thymine reversibly inhibits action of antibiotics like trimethoprim group.
Thickness	If thickness of medium is more, growth of organism is less and vice versa.

Use antibiotic discs of 6 mm diameter, with proper space between the discs kept in AST.

Store supply of antimicrobial discs at -20˚C

Use correct content of antimicrobial agent per disc

Use Mueller-Hinton medium for antibiotic sensitivity determination.

To avoid errors

Use appropriate control cultures.

Periodic service and calibration of instruments required in AST.

Use standard methodology for the test

Checking of expiry date of discs before use

Training of personnel with the correct technique.

Maintaining of proper temperature of all the equipments required in AST.

REFERENCES

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R. C. Dubey and D. K. Maheshwari. (2024). A Textbook of Microbiology, 5th Edition,ISBN:978-93-550-1527-3.

THANK YOU