



BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024

Tamil Nadu, India.

Programme: M.Sc., Biomedical Science

Course Title : Clinical Microbiology

Course Code : 18BMS48C15

Unit-I

**TOPIC: Antibiotic Sensitivity Disc, Testing
Procedure and their Quality Control**

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**ANTIBIOTIC SENSITIVITY DISC
TESTING PROCEDURE AND THEIR
QUALITY CONTROL**

Bacteria exhibit great strain variations in susceptibility to antimicrobial agents



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

Antimicrobial
Susceptibility
Testing
(AST)

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

Antimicrobial Susceptibility Testing

Diffusion method

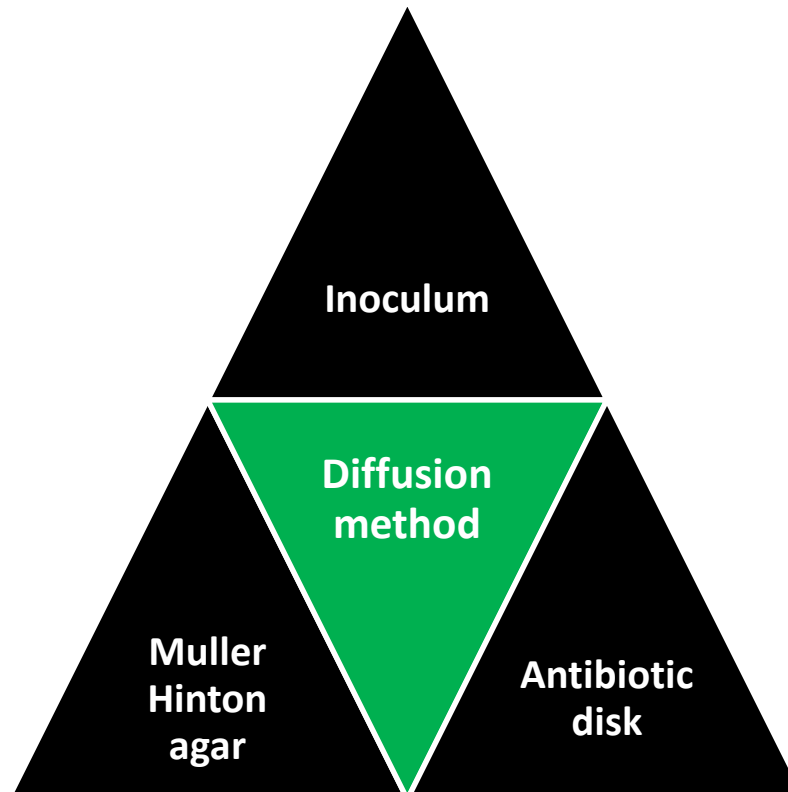
- Kirby-Bauer
- Stokes

Dilution method

- Broth Dilution
- Agar Dilution

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

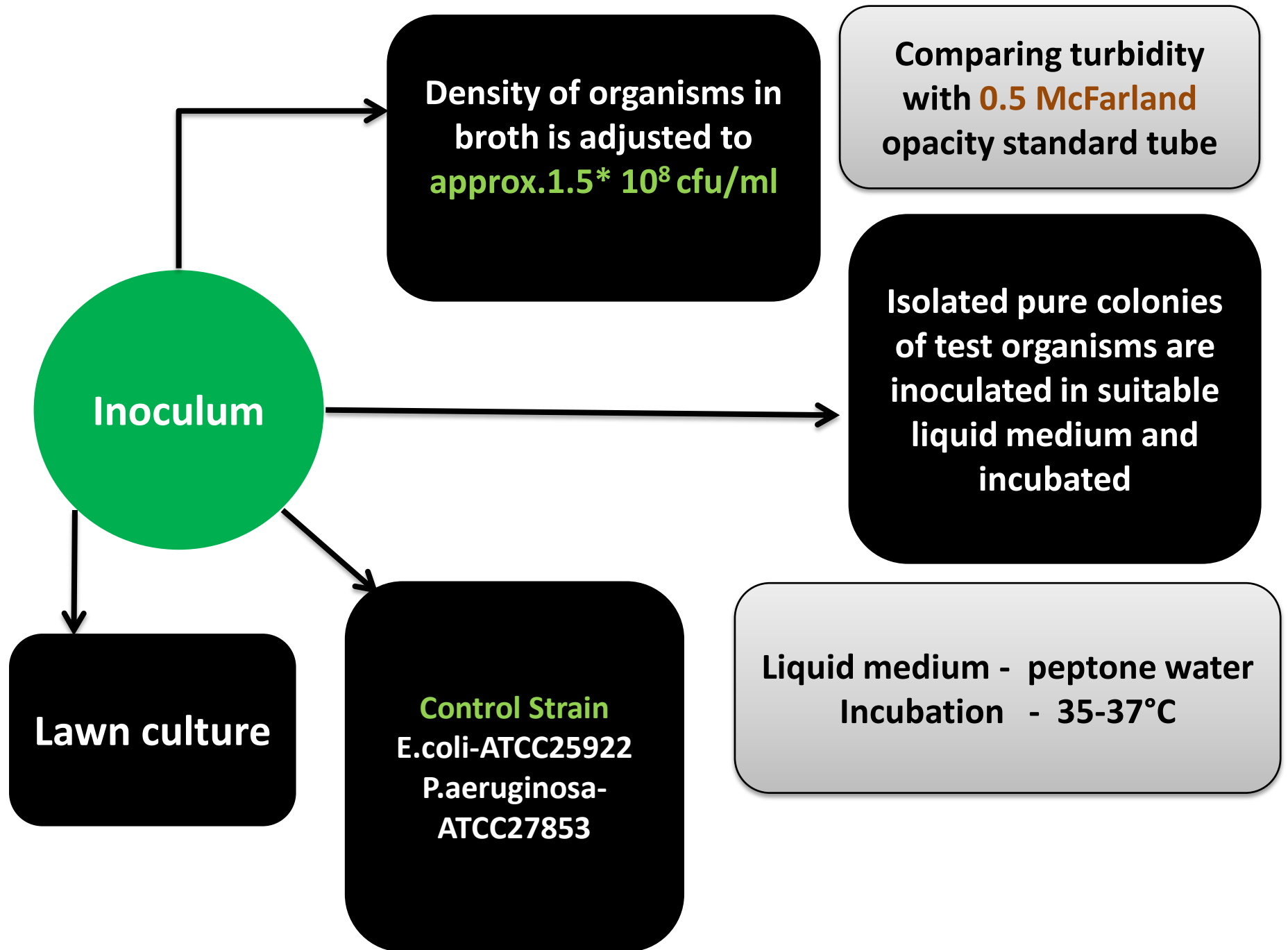
**Muller Hinton
Agar**

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 *Staphylococcus aureus*)**



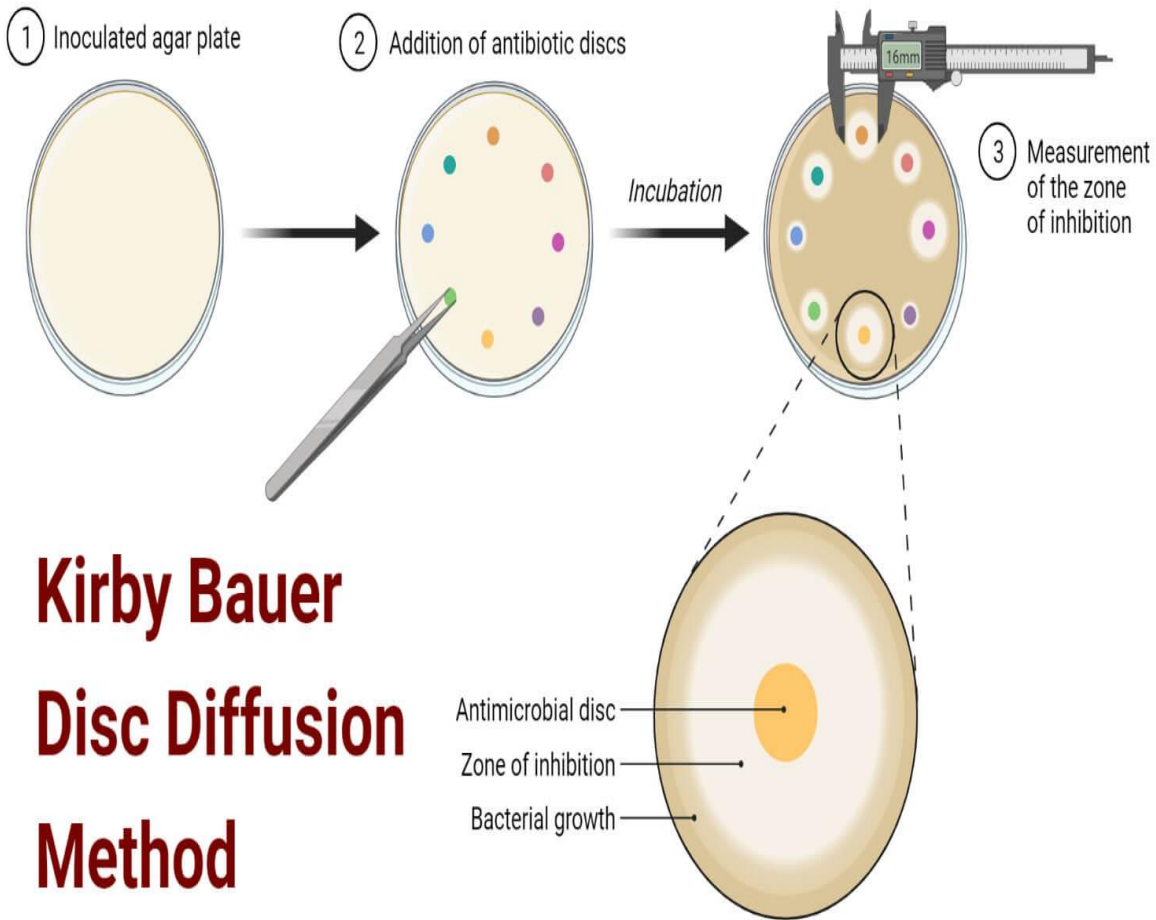
Whatman no : **1**
Diameter - **6mm**

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

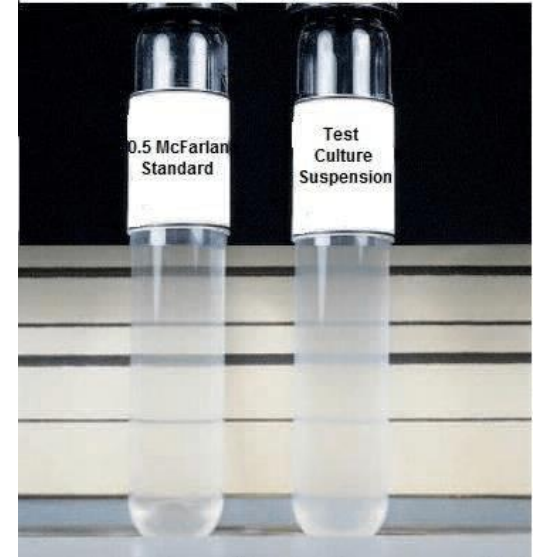
**Antibiotic
disk**

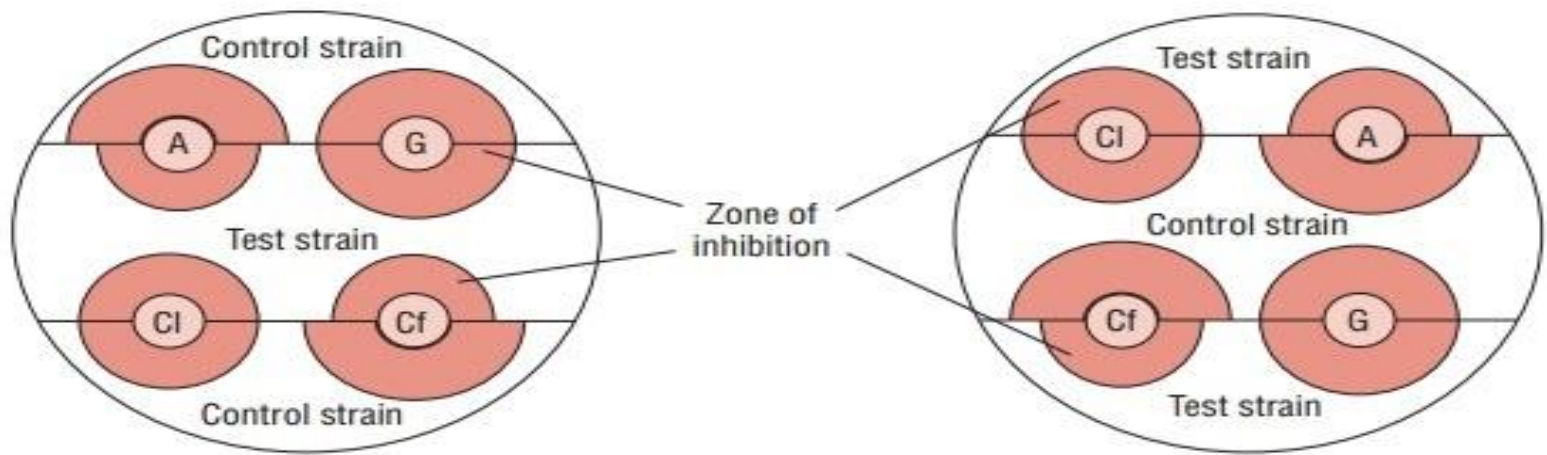
Antibiotic delivered
with **20 gauge wire**
loop

Should be stored in
sealed containers
with **dessicants**



Kirby Bauer Disc Diffusion Method

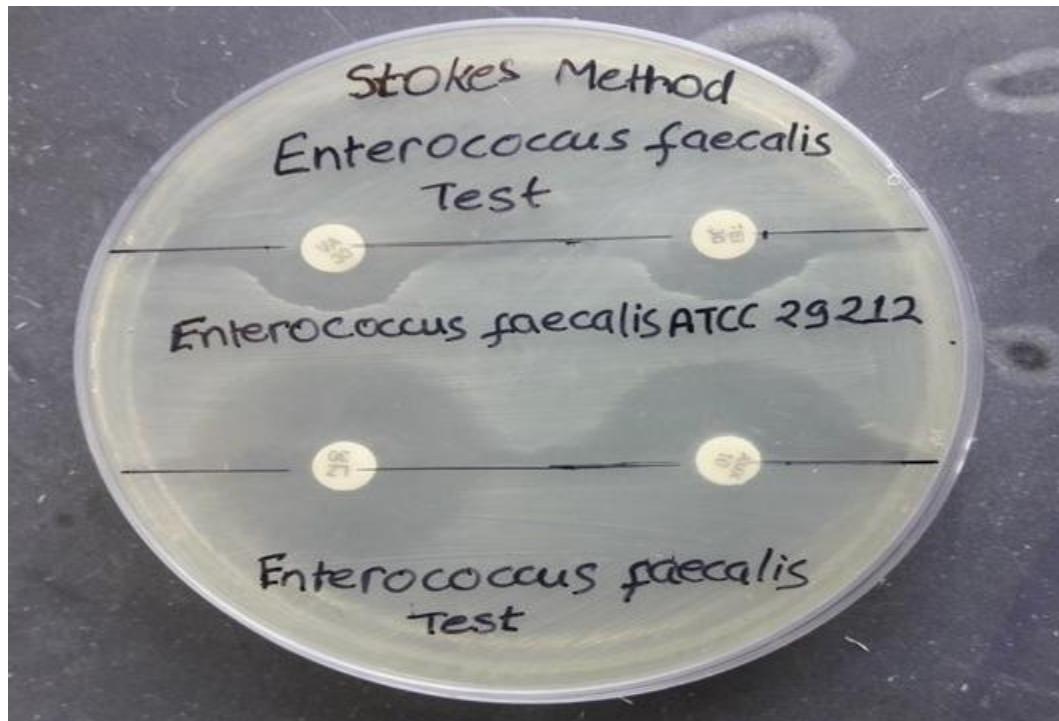




Stokes disc diffusion method

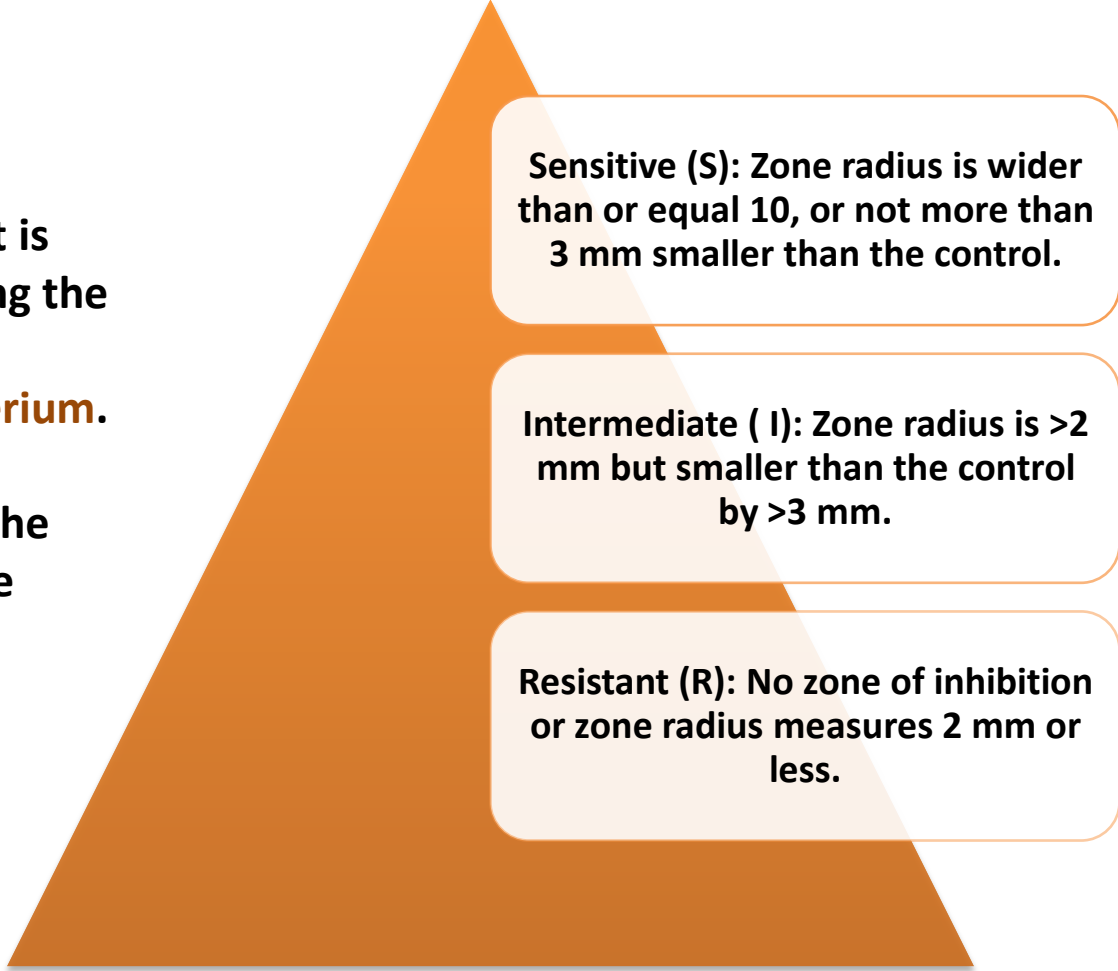
Modified Stokes disc diffusion method

Schematic diagram showing Stokes method of antibiotic sensitivity.



Reporting in Stokes Method

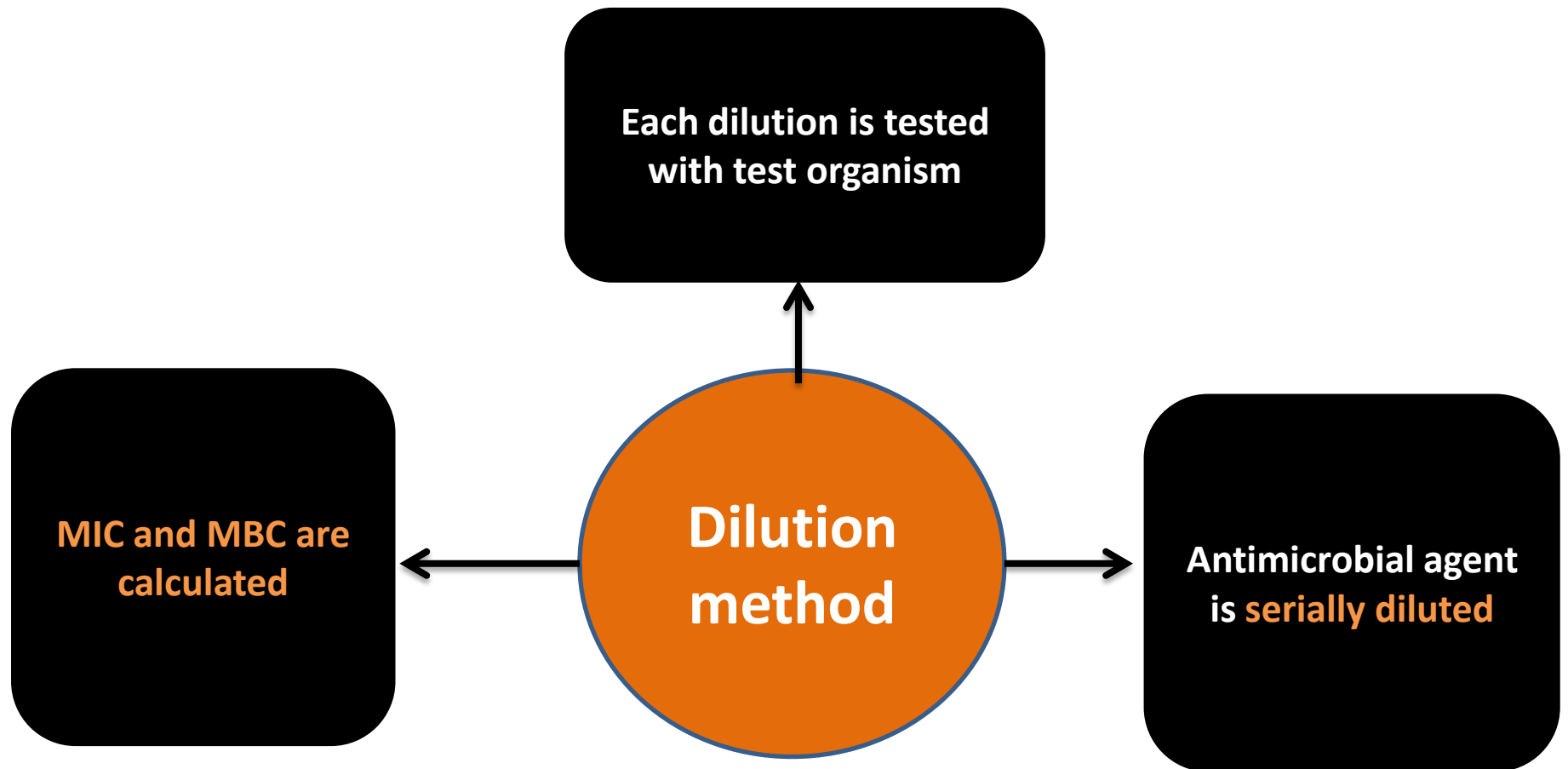
- The sensitivity report is prepared by comparing the **zones of inhibition of control and test bacterium.**
- The radius of the inhibition zone from the edge of the disk to the edge of the zone is measured.



Sensitive (S): Zone radius is wider than or equal 10, or not more than 3 mm smaller than the control.

Intermediate (I): Zone radius is >2 mm but smaller than the control by >3 mm.

Resistant (R): No zone of inhibition or zone radius measures 2 mm or less.



- **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

Test organism is inoculated in tubes containing serial dilutions of an antibiotic

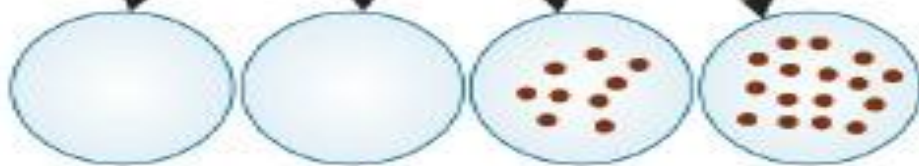
Antibiotic conc ($\mu\text{g/mL}$)

64 32 16 8 4 2 1 Control



Subcultures on
solid media

Turbidity indicates growth

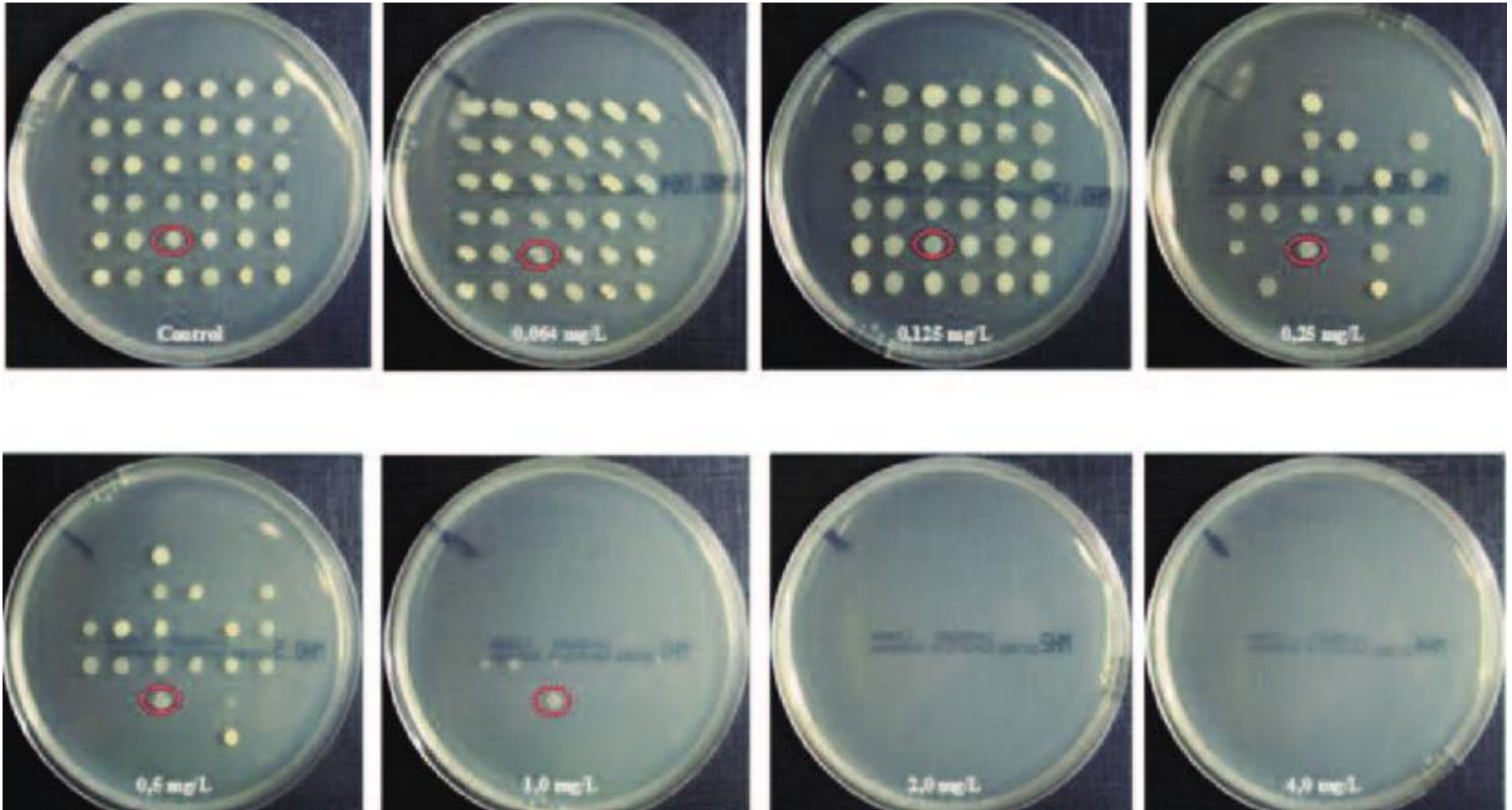


No growth

Growth

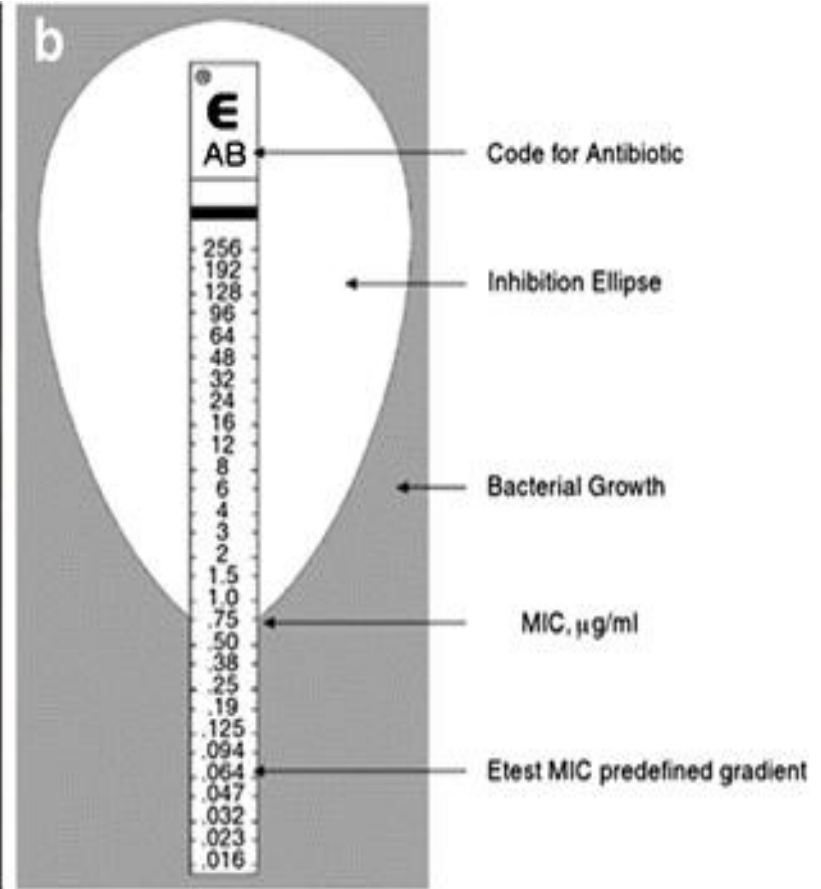
MIC = 8 $\mu\text{g mL}$
MBC = 32 $\mu\text{g mL}$

Agar Dilution Method



Epsilonometer 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

Ananthanarayan and Paniker's. (2023). Text book of Microbiology, 12th Edition. Universities Press Private Ltd., ISBN:978-93-93330-01-7.

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

THANK YOU