

# Antibiotic sensitivity testing

**\* Antibiotic sensitivity testing is done to know the response of a particular organism to a drug or combination of drugs.**

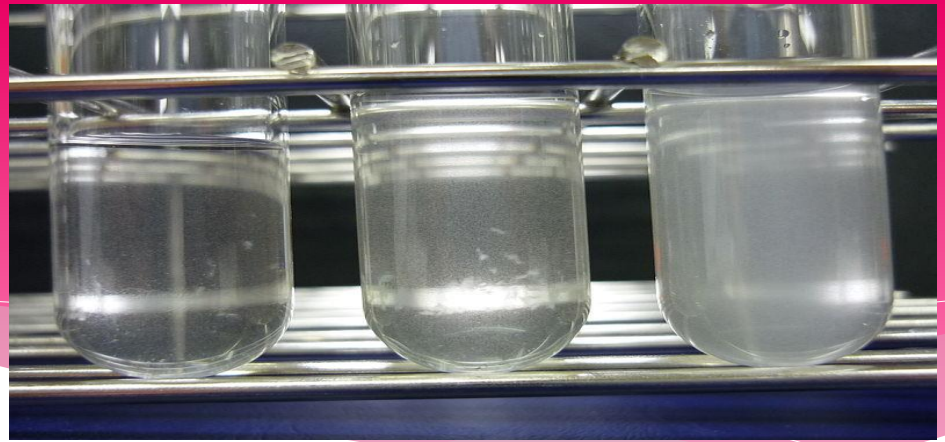
**\* It can be carried out by following methods.**

- 1. Disc diffusion method**
- 2. Dilution method (Broth or agar dilution methods)**
- 3. Automated method ( Vitek / Phoenix / microscan )**

# Disc diffusion method:

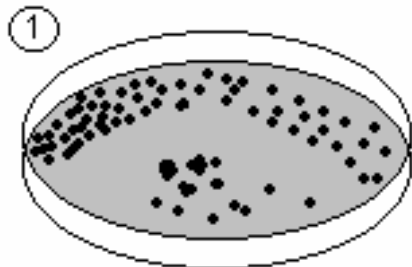
- \* **Principle:**
- \* **Plate of a suitable agar is flooded with suspension of organism and antibiotic discs are applied on it.**
- \* **Plates are incubated for 18-24 hours and zone of inhibition of growth around each drug disc are seen and compared with standards.**

# Method:



- \* Suspension of organism is prepared in peptone water by similar colonies from agar plate.
- \* Turbidity of peptone water is compared with Mcfarland's Barium Sulphate suspension.
- \* Suspension of organism is flooded on dried plate of agar by lawn culture method with sterile swab.
- \* Antibiotic discs are applied on it.
- \* Plates are incubated for 18-24 hours.
- \* Zone of inhibitors around the disc is to be observed, after incubation period.

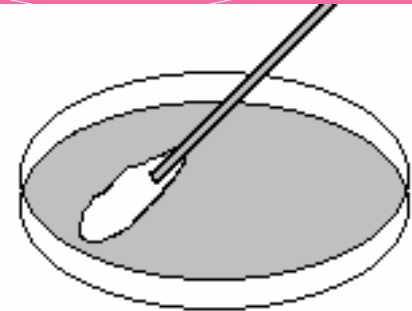
## Procedure for disk diffusion testing



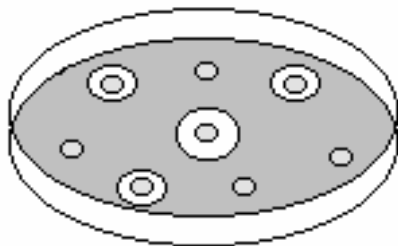
①  
2-3 identical colonies are picked from the plate and transferred to the broth



②  
The tube is incubated for the bacteria to grow.  
The inoculum density is standardized using McFarland standard

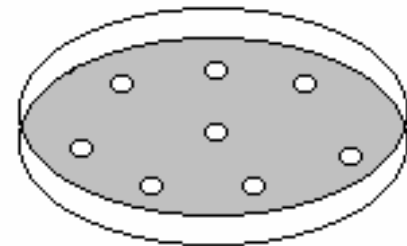


③  
A cotton swab dipped in the inoculum suspension is swabbed over the entire surface of agar to give a lawn culture.



⑤  
Zone diameter around the disk are measured and result read from Kirby Bauer chart

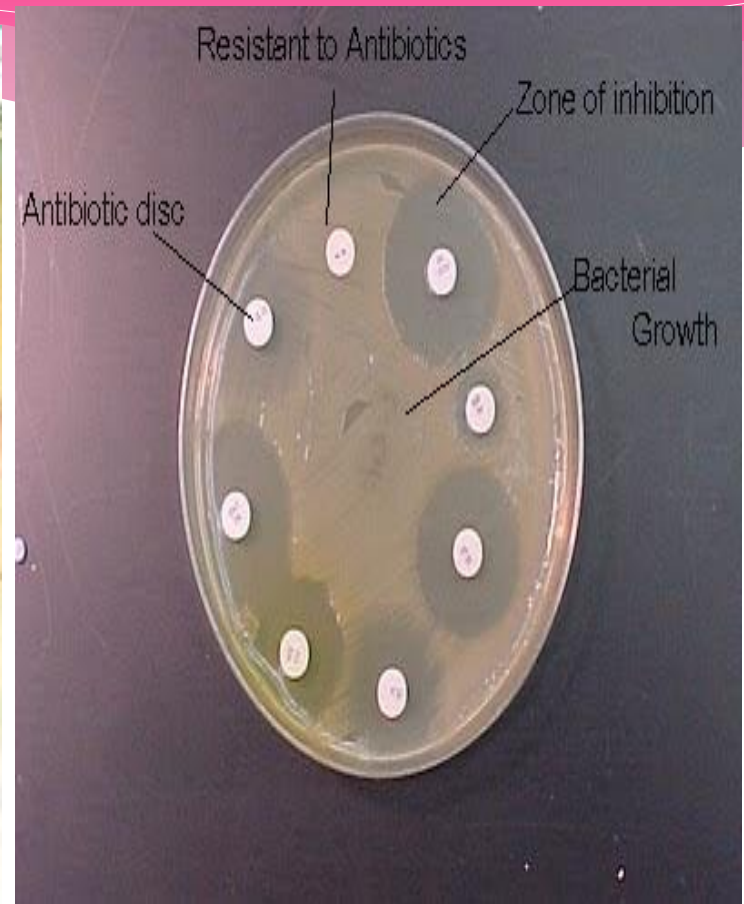
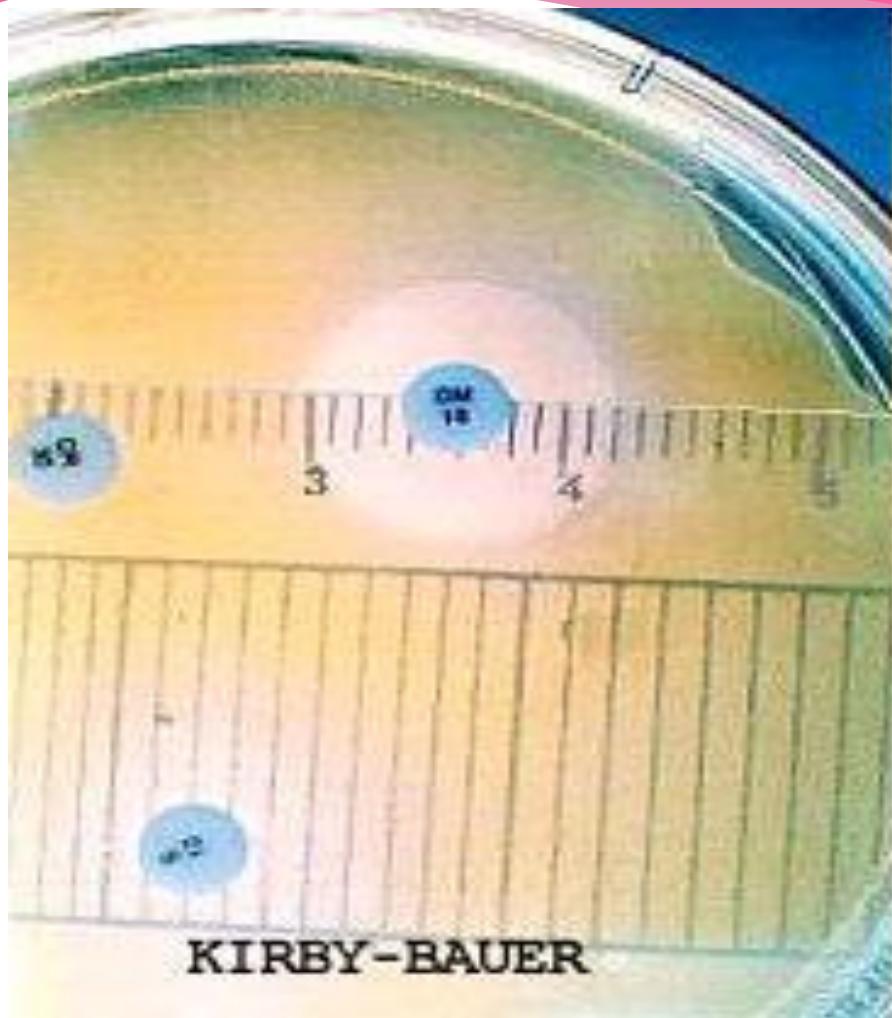
← Plate incubated at 37C overnight



④  
Filter paper disks containing known antibiotic in known concentration is placed on the surface of inoculated agar.

# Results:

- \* Zone of inhibition of growth - as comparable to standard - Bacteria **sensitive** to that drug.
- \* Zone of inhibition of growth is less than that of standard - Bacteria **resistant** to particular drug.
- \* No Zone of inhibition - Bacteria **resistant**.



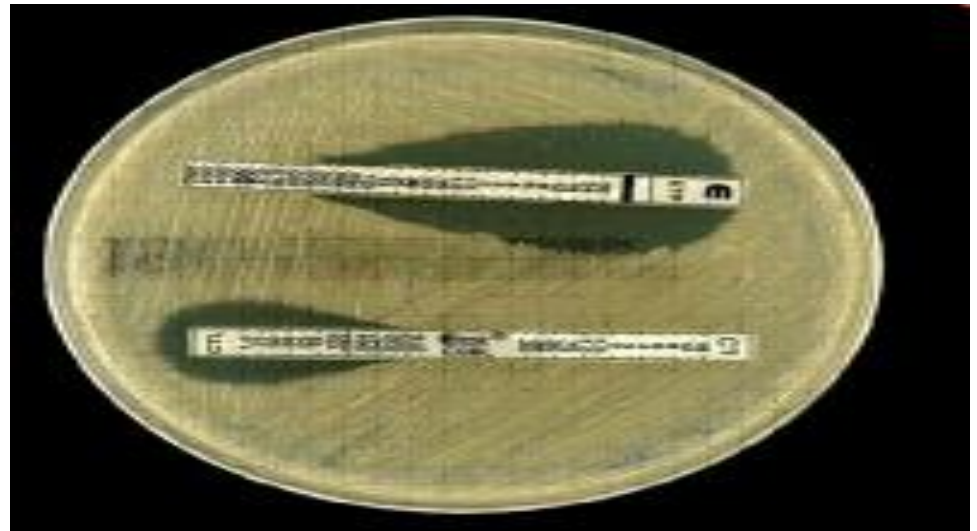
Name plating media used for disc diffusion method.

- \* **Müller-Hinton agar**
- \* **Nutrient agar**




# Name different method for testing antibiotic sensitivity pattern.

- \* **Disc diffusion**
- \* **Dilution-broth and agar**
- \* **Diffusion and dilution**
- \* **Automation**



# What do you mean by MIC & MBC?

- \* **Minimum inhibitory concentration (MIC)**, in microbiology, is the lowest **concentration** of an **antimicrobial** that will inhibit the visible **growth** of a micro organism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to **confirm** resistance of micro organisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents.

- 
- \* **The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium.**

***Practical 11 (B)***

***Laboratory animals***

# **General uses of the animals**

- 1. For pathogenicity tests.**
- 2. Isolation of specific organisms.**
- 3. Preparation of specific antisera.**
- 4. Attenuation and exaltation of organisms.**
- 5. Demonstrating the use of certain hormones.**
- 6. Experimental purposes.**

# **Routes of inoculation**

- a. Skin scarification.**
- b. Intradermal inoculation.**
- c. Subcutaneous inoculation.**
- d. Intramuscular inoculation.**
- e. Intravenous inoculation.**
- f. Intraperitoneal inoculation.**
- g. Inoculations in to the special sites  
e.g. intrathecal, intratracheal,  
conjunctival etc**

# **Material inoculated**

- **Body fluids and fluid exudates e.g. blood, urine, CSF, pus, aspirated fluids etc.**
- **Tissue emulsion e.g. of lymph node.**
- **Bacterial cultures.**

*Chief uses of  
the individual  
animals*



# Rabbits



- ❖ **For pregnancy test (Freidman's test) only females rabbit used.**
- ❖ **For preparing antisera. Blood is collected from marginal vein of ear and by cardiac puncture.**
- ❖ **For diagnostic pathogenicity tests.**

# Guinea pig



- ❖ **For isolation of *M. tuberculosis*.**
- ❖ **For pathogenicity test of *C. diphtheria*, *B. anthracis*, *Y. pestis* etc.**
- ❖ **For obtaining complement (used in complement fixation reaction)**
- ❖ **For differentiation of Rickettsial diseases.**
- ❖ **Sereny's Test - If Guinea pig's conjunctiva is instilled with EIEC Strain of *E. coli* will produce kerato conjunctivitis in the eye.**

# Albino rat



- ❖ **Same as guinea pig and for special experimental work.**
- ❖ **To differentiate *Y. pestis* from *Y. pseudotuberculosis*.**
- ❖ **Very useful in nutritional research work.**

# White mice



❖ **For isolation of pneumococci.**

❖ **For pathogenicity tests.**

❖ **For pregnancy test (Ascheim-Zondiak Test)**



# Rhesus monkey

- ❖ **For isolation of viruses in tissue culture. Kidney tissue is used.**



# Sheep

- ❖ **For obtaining red blood cells used in the complement fixation test and Paul Bunneltest.**
- ❖ **Whole blood for preparation of blood agar.**



# Armadillo

- ❖ **Nine banded armadillo is inoculated with lepra bacilli for isolation of lepra bacilli.**
- ❖ **For preparation of lepromin "A" antigen.**



Thank

you