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MICROBIOLOGICAL ASSAY

Principal and Methods of different **microbiological assay** :

- Method for standardization of **antibiotics** , **vitamins** and **amino acids**
- **Assessment of a new antibiotics**

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Microbiological Assay :

Also called as **microbial assay**

- A microbiological assay defined as quantitative or qualitative determination of chemical compounds (antibiotics , vitamins and amino acids) with the use of microorganism .
- It is necessary to assay antimicrobial

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agent , for determination of **potency** of a drug in animals or man and monitoring antimicrobial chemotherapy .

Microbiological Assay of Antibiotics (method for standardization of antibiotics):

→ It is based upon a **comparison** of the inhibition of growth of micro – organism by measured concentration of the antibiotics under examination

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(test antibiotics) with the known concentration of a standard known antibiotics preparation .

★ Two general methods are usually employed ,

- 1) Cylinder – plate OR Cup – plate Method
- 2) Turbidimetric OR Tube assay Method

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Preparation For Microbiological Assay :

- Preparation of media and buffer solution .
- Selection of microorganism
- Preparation of test and standard solutions of antibiotics .

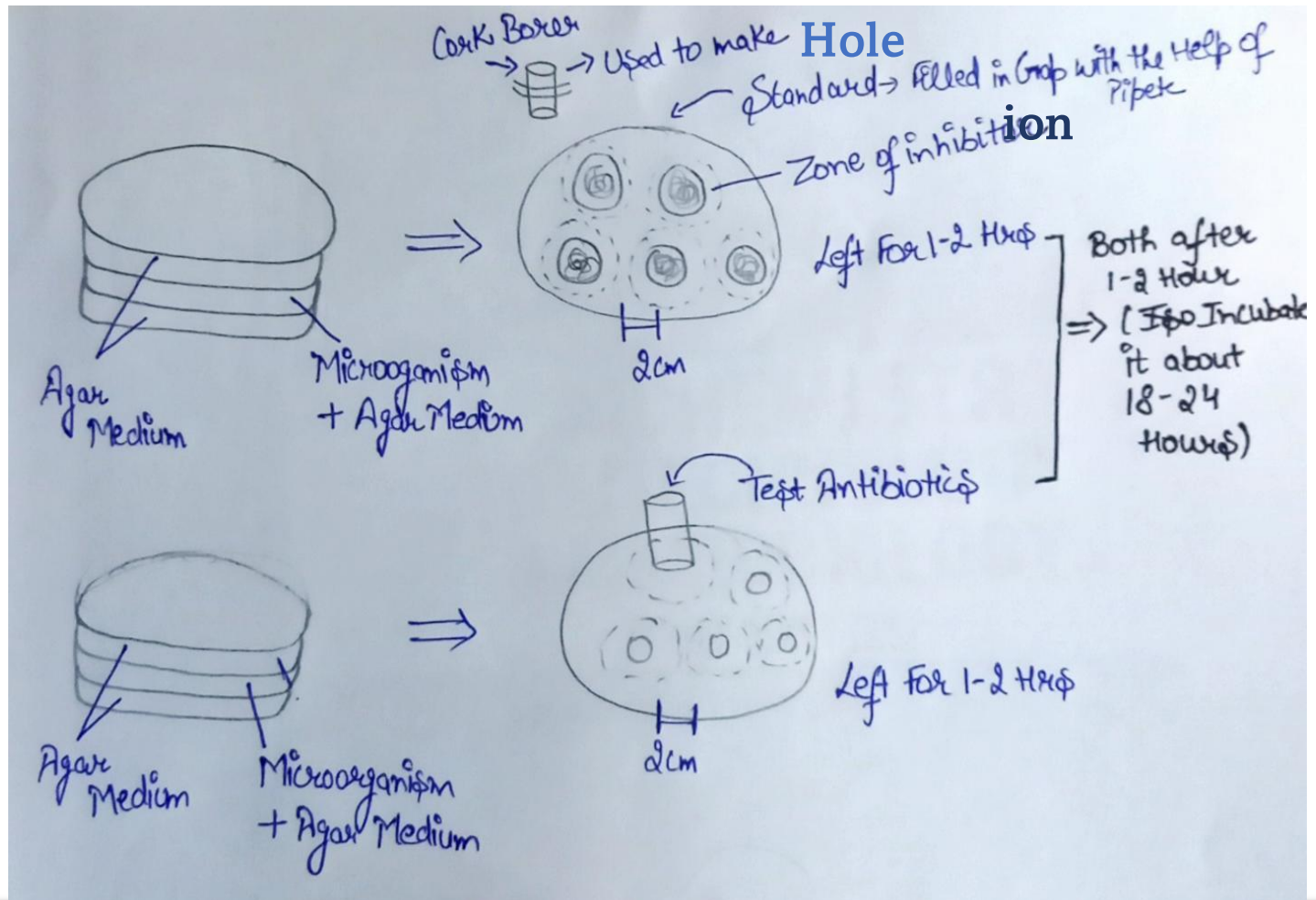
{ 3 layer – 1 , 3 (Agar medium) }

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→ Microorganism E.g. – *Bacillus pumilus* ,
Staphylococcus epidermis etc .

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1) Cylinder – plate OR Cup – plate Method



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★ Comparison :

→ Instandard Solution :

We provide Favorable temperature ,
Favorable Agar solution



For growth of bacteria , but in
standard solution Antibiotic inhibit
growth of bacteria and this zone is
called **zone of inhibition**

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→ **In Test Antibiotics :**

If some zone diameter appear

E.g. – suppose 2 cm in standard and 2 cm comes in Test (kill microbes)

Then it's equal to standard and test antibiotic is okay and If not in equal range then

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Potency of test antibiotic is not good

Method 2 :

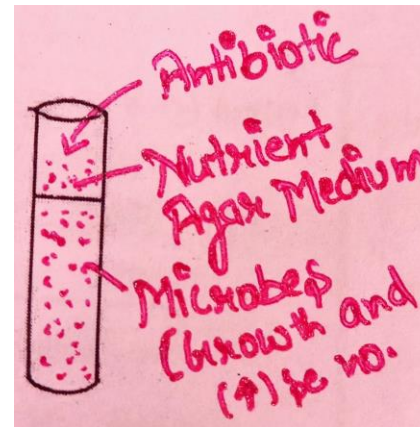
Turbidimetric OR Tube assay Method :

- This method depends upon the growth of microbial culture in a uniform solution of the antibiotics in a fluid medium that is favorable to its

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rapid growth in the absence of the antibiotics .

→ It has a shorter incubation periods for the growth of test microorganism (4 to 5 hours) .



Test Tube

→ **Because
of agar
Medium**

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Antibiotic → Inhibit Growth

Agar Medium → Promote growth of microorganism

★ **As a result (Turbidity appears)**

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So this method is not recommended for cloudy or turbid preparation

Turbidity



More Turbidity



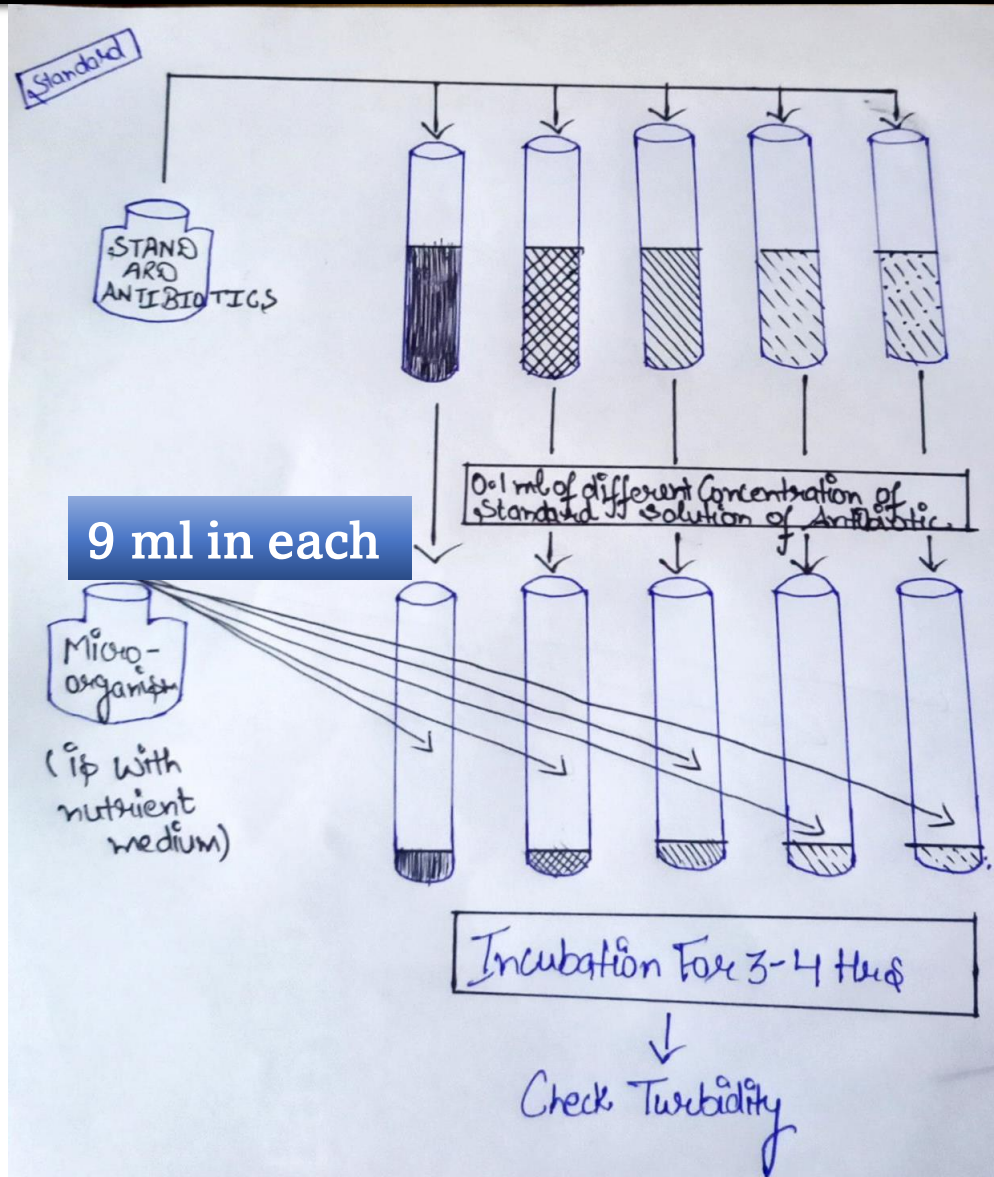
**It means more
presence of
microorganism**

Less Turbidity



**It means Less
presence of
microorganism**

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At the same time →

Three control tubes , one containing the inoculated culture Media , second treated with 0.5 ml of dilute formaldehyde solution (blank) and third containing un – inoculated culture Media are prepared

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Same For test →

If test turbidity increases as compared to standard



Then test is not prefect (because more turbidity more microorganism)