MICROBIOLOGY

UNIT 4 NOTES

ACEPTIC AREA

- LAMINAR FLOW EQUIPMENTS
- SOURCES PF CONTAMINATION
- METHOD OF PREVENTION
- CLEAN AREA CLASSIFICATION

MICROBIOLOGICAL ASSAY

- ANTIBIOTICS
- VITAMINS
- AMINO ACIDS

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ASCEPTIC AREA

- An Asceptic Area is a controlled environment designed to maintain sterility during the production, preparation or handling of materials, particularly in industries like pharmaceuticals, biotechnology and healthcare.
- The main goal of asceptic area is to minimize the risk of contamination by microorganisms, particles or other impurities.

CHARACTERISTICS OF ASCEPTIC AREA

① Controlled Environment

- The area is designed with strict controls over temperature, humidity air quality and pressure.
- Positive air pressure is maintained to prevent external contamination.

② Air Filtration System

- It utilizes High-Efficiency Particulate Air (HEPA) filters to remove airborne particles and microorganisms.
- Laminar airflow system can be used to ensure uniform airflow and minimize contamination.

③ Personnel Hygiene & Gowning

- Personnel must undergo strict gowning procedures, including wearing sterile suits, gloves mask & shoe covers.

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④ Environmental Monitoring

- Continuous monitoring of microbial & particulate contamination is performed.
- Parameters like air particle counts, surface sterility, temperature, humidity are routinely checked.

⑤ Sterile Materials & Equipments

- All equipments, tools and materials are sterilised before entering the area, often through autoclaving or chemical sterilization.

⑥ Regular Cleaning & Disinfection

- Surfaces are cleaned with validated disinfectants and sterilants to prevent microbial contamination.
- Cleaning is performed according to standard operating procedures.

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Classification Of Asceptic Area

Asceptic ~~area~~ area can be classified into 4 grades :

- Grade A
- Grade B
- Grade C
- Grade D

GRADE A

- These are critical zones where sterile products or materials are exposed to the environment, such as during aseptic filling or manufacturing.
- They are used for activities requiring highest level of sterilization.

GRADE B

- It is the background for areas housing Grade A zones.
- It supports aseptic operations, providing a controlled low-contamination environment.

GRADE C

- It is the area used for less critical activities in sterile manufacturing such as preparation of solutions or components.

GRADE D

- It is the area used for non-critical operations, such as handling of raw materials before sterilization.

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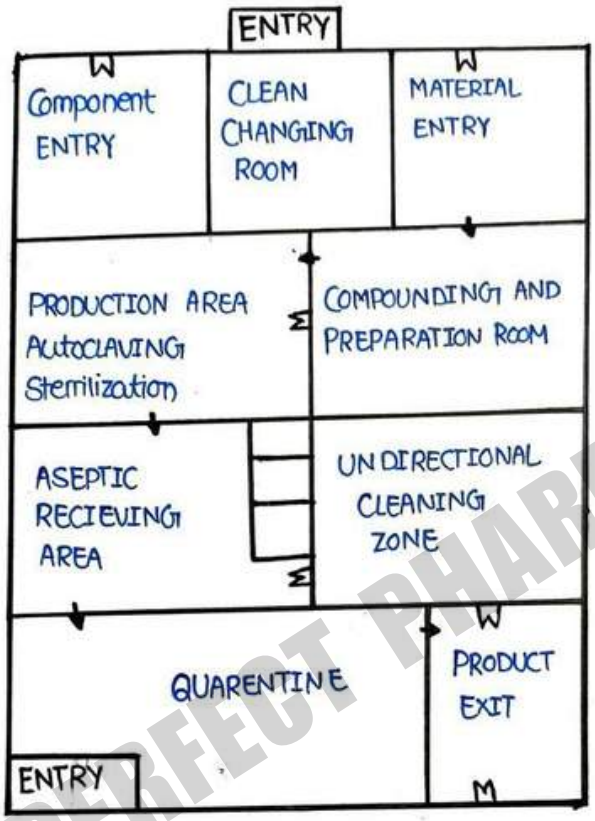

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LAYOUT OF ASEPTIC AREA

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LAMINAR FLOW EQUIPMENTS

- They are also known as Laminar air flow/ Laminar flow Cabinet Tissue Culture Hood / Clean Benches.
- Laminar Air Flow Principle was first developed in early 1960.
- The air filtered from Laminar air flow is claimed to be 99.99% free from microbial contamination.
- It does not provide filtration to vapour or Gases.

How It Works

- Laminar Air Flow Equipments used HEPA & ULPA filters to trap airborne particles.
- An internal fan forces air through the filter, creating a steady low turbulance flow that maintain a contamination free environment by pushing particles out of the workspace.

Application

- Medical & Pharmaceutical Labs
- Electronics Manufacturing
- Food Industries
- Research Laboratories

Types

① Horizontal Laminar Flow Cabinet
② Vertical Laminar Flow Cabinet

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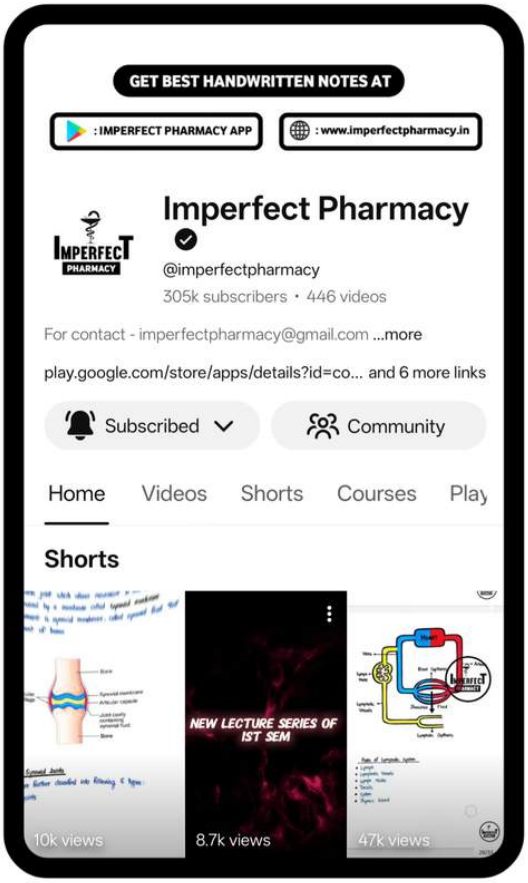

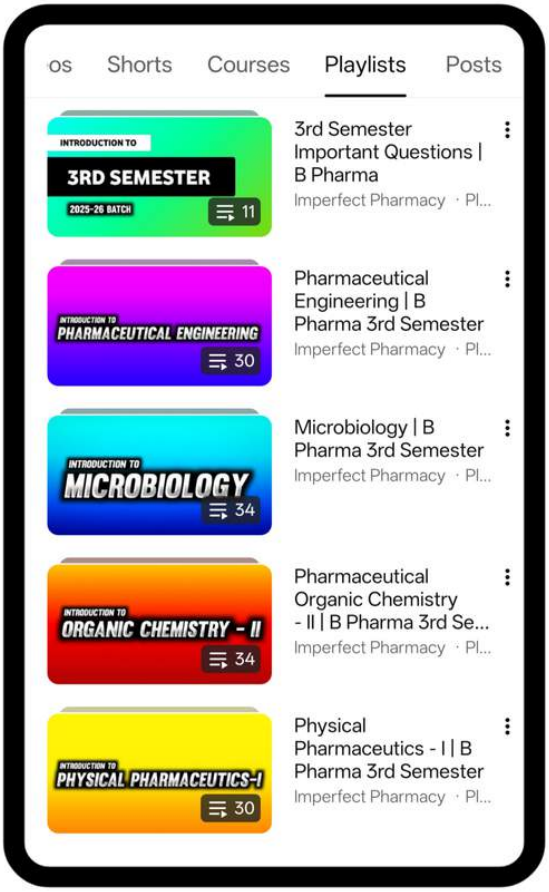

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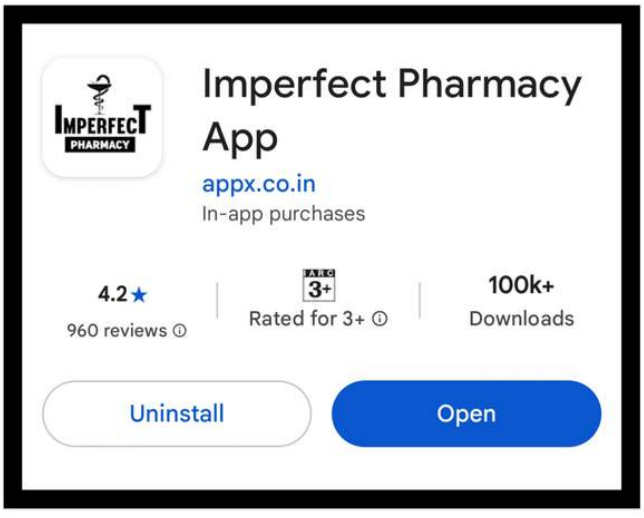
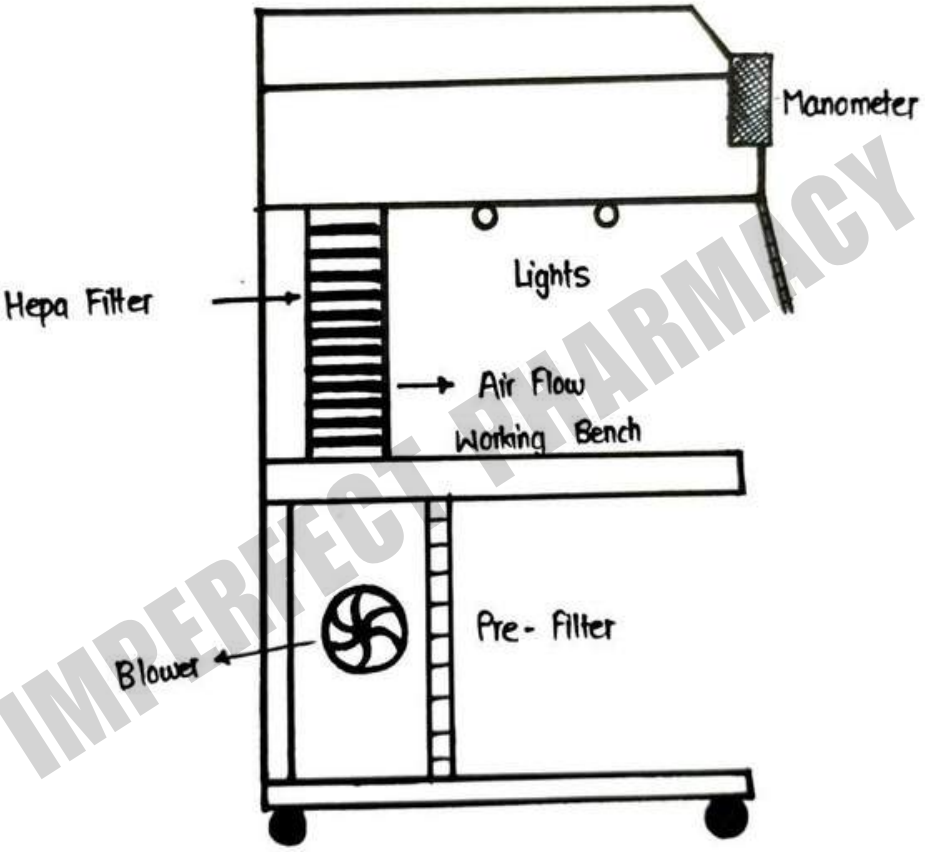


HORIZONTAL LAMINAR AIR FLOW

- Horizontal Laminar flow Cabinet recieve thier name due to the direction of of air flow which Comes from above but then Change direction and is processed across the work in a Horizontal direction.
- The Constant flow of filtered air provides material and product Protection.



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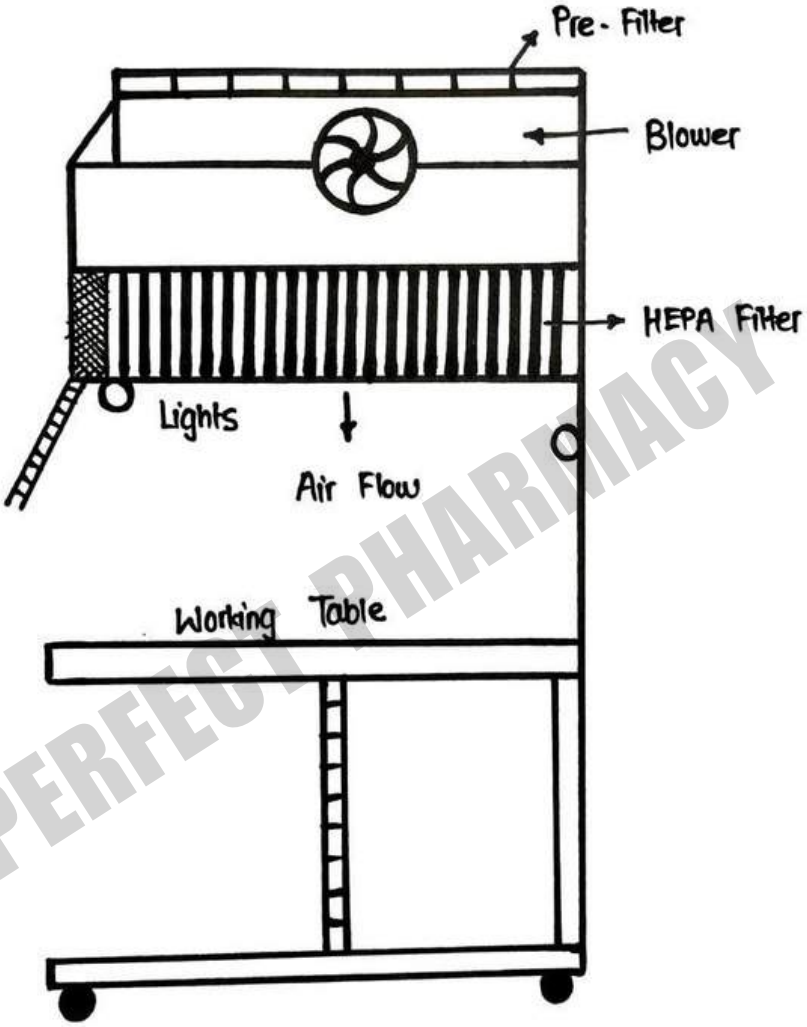
VERTICAL LAMINAR FLOW

- In vertical laminar flow , air flows vertically from top to bottom .
- Air is filtered through HEPA



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SOURCES OF CONTAMINATION & THEIR PREVENTION

- In Aseptic Area, maintaining a sterile environment is crucial to prevent contamination.
- Below are some primary sources of contamination & their prevention in aseptic areas.

① Atmosphere
② Operators
③ Raw Material
④ Equipments
⑤ Water Supply

① ATMOSPHERE

- Air is the major source of contamination as it contains dust particles, microbes, spores & various other airborne particles.
- It can be reduced by using chemical disinfectant, HEPA filters, maintaining positive pressure & using Laminar Flow Equipments.

② OPERATOR

- Microorganisms from skin, hair and clothing of the operator are potent source of microbial contamination.
- It can be reduced by maintaining proper hygiene & wearing sterile garments including gloves, masks, hair covers & gowns

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③ RAW MATERIALS

- Raw materials can be responsible for high proportion of microbial contamination if not sterilized.
- It can be reduced by ensuring that all materials entering the aseptic area are sterilized & disinfectant.

④ EQUIPMENTS

- The equipments and utensils used in processing, holding, transferring and packaging are also common source of contamination in aseptic area.
- It may be reduced by sterilization or disinfection by heat, gaseous agents or chemicals.

⑤ Water Supply

- Contaminated water can introduce bacteria or chemical contaminants if used in processes like rinsing or cooling.
- It can be reduced by using distilled water

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CLEAN AREA CLASSIFICATION

- A Clean area refers to a designated space that is maintained to be free from contaminants, dirt & pollutants.
- The classification of clean area typically follows standards set by organizations such as ISO (International Organization for Standardization) & federal standard 209 E.
- The most widely used classification system is ISO 14644-1

ISO Class	Particles / Cubic Metre ($\geq 0.1\mu m$)
ISO 1	10
ISO 2	100
ISO 3	1,000
ISO 4	10,000
ISO 5	100,000
ISO 6	1,000,000
ISO 7	Not Specified
ISO 8	Not Specified
ISO 9	Not Specified

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MICROBIOLOGICAL ASSAY

- A Microbiological assay is a method used to determine the concentration or potency of a substance by observing its effect on microorganisms.
- The technique is commonly employed to assess compounds such as antibiotics, vitamins and amino acids.
- The assay involves microbial growth responses to varying concentrations of the substance, allowing for both qualitative and quantitative analysis.

MICROBIAL ASSAY IN OUR SYLLABUS

- Antibiotics
- Vitamins
- Amino Acids

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MICROBIOLOGICAL ASSAY OF ANTIBIOTICS

- Antibiotics are class of drugs used to treat infections caused by bacteria.
- Microbiological assay of antibiotics is a laboratory method used to determine the potency & concentration of antibiotics substances by observing their effects on specific microorganisms.

PRINCIPLE

- The principle behind assay of antibiotic is based on inhibition of growth of ~~anti~~ specific microorganisms in proportion to the concentration of the antibiotic present.
- By comparing inhibition caused by an standard antibiotic with the sample being tested, potency of test antibiotic can be determined.

REQUIRMENTS

- Test solution
- Standard solution
- Culture Medium

TEST ORGANISM FOR DIFFERENT ANTIBIOTICS

ANTIBIOTICS	TEST MICROORGANISMS
Amikacin	Staphylococcus aureus
Amphotericin B	Saccharomyces cerevisiae
Bleomycin	Mycobacterium smegmatis
Doxycycline	Staphylococcus aureus
Rifampicine	Bacillus subtilis

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Composition of Media Used For Assay of Antibiotics

- Peptone : 6.0 g/L
- Pancreatic digest of casein : 4.0 g/L
- Yeast Extract : 3.0 g/L
- Beef Extract : 1.5 g/L
- Dextrose : 1.0 g/L
- Agar : 15.0 g/L

Methods For Assay Of Antibiotics

Pharmacopoeias suggest following two antibiotic assay methods :

① Turbidimetric or Tube Assay Method
② Cup-Plate or Cylinder Plate Method

① Turbidimetric Method

- This method involves introducing varying concentrations of the antibiotic into a liquid broth medium inoculated with microorganism.
- The mixture is incubated, allowing the antibiotic to interact with the bacteria.
- Bacteria growth leads to increased turbidity of broth, which can be measured using a spectrophotometer.
- By comparing the turbidity readings of standard & test samples, potency of test samples can be determined.

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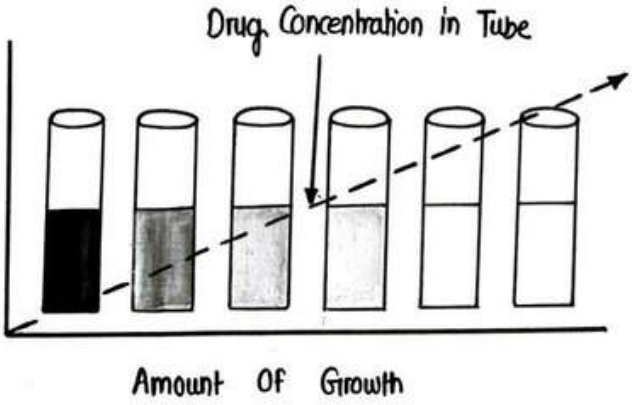
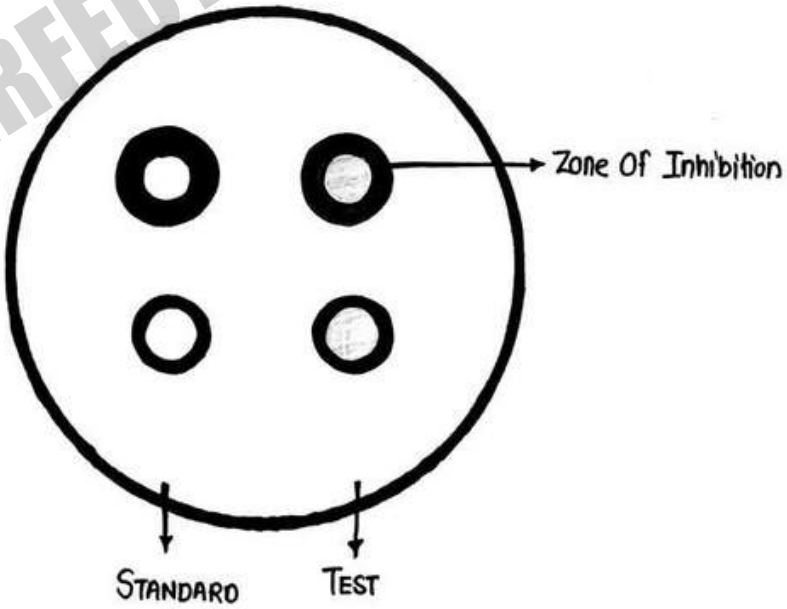


② Cup Plate Method

- In this technique, a solid agar medium is uniformly inoculated with the test microorganism.
- Cylindrical cups or wells are then introduced into the agar. known concentration of standard & test antibiotics are placed in cylinders.
- The potency of unknown sample is determined by comparing the size of its zone of inhibition with that of standard.



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MICROBIOLOGICAL ASSAY OF VITAMINS

- The microbiological assay of vitamins is a method used to determine the concentration of vitamins based on growth response of specific microorganisms.
- The assay is widely employed for water soluble vitamins such as B-complex vitamins (e.g. B_1, B_2, B_6, B_{12}) and folic acid.

PRINCIPLE

- Microorganisms have specific vitamin requirments for growth.
- In this a test sample is provided as a nutrient source for the microorganisms.
- The growth of microorganisms is proportional to amount of vitamin present. By comparing growth response of test with that of standard with a known concentration, quantity of vitamin in sample can be determined.

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PREPARATION OF BASAL MEDIUM

- The basal medium for microbiological assay of vitamins is a complex mixture of nutrients the provides everything the test organism needs to grow
- Key components of Basal Medium (for Vitamin B_{12})

* Carbon Source : Glucose, Dextrose
* Nitrogen Source : Casein Hydrolysate, Ammonium Salts, Yeast Extract
* Buffering Agent : Usually phosphates
* Trace Elements : Iron, Magnesium, Zinc etc.
* Growth Factors

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PROCEDURE

- Clean 10 test tubes and add , 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml, 3.5 ml, 4 ml, 4.5 ml & 5 ml respectively of standard Vitamin B12 (Cyanocobalamin) solution.
- To each test tube add 5 ml basal medium solution.
- Adjust final volume to 10 ml using water
- Now Take other 4 test tubes & add 1 ml, 2 ml, 3 ml & 4 ml respectively of test solution to be assayed.
- To each test tube add 5 ml basal medium stock solution.
- Adjust final volume to 10 ml using water
- Sterilize all test tube in autoclave at 121°C for 5 minutes.
- Now add 1 drop of bacterial culture to each test tube.
- Incubate them for 64 to 72 hours at temperature range of 30-37°C.

MEASURMENT OF GROWTH

After incubation growth of microorganism can be measured & compared using following two methods:

① Turbidimetric Method
② Titrimetric Method

APPLICATION

- Food Analysis
- Pharmaceutical Analysis
- Clinical Diagnosis
- Research

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MICROBIOLOGICAL ASSAY OF AMINO ACIDS

The Microbiological assay of amino acids is a method used to quantify the concentration of amino acids in a sample by measuring the growth response of microorganisms that require these amino acids

PRINCIPLE

- Certain microorganisms require specific amino acid for growth.
- A Growth medium is prepared in which test & standard samples are added.
- The growth of microorganism that require these amino acids measured.
- The concentration of amino acids determined by comparing the growth of micro organisms in test and standard sample.

PROCEDURE

- Preparation of Media : A basal media lacking the amino acid being tested is prepared.
- Selection of Microorganism : A Microorganism that requires the specific amino acid is chosen.
  for Example : Lactobacillus arabinosus for Lysine
  : E. Coli for Tryptophan
- Inoculation : The microorganism is inoculated into the medium containing the test and standard solution of amino acids.

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- **Incubation** : The cultures are incubated under optimal growth conditions.
- **Measurment Of Growth** : The growth is measured, typically by :
  - Optical Density (Turbidity)
  - Acid Production (change in pH)
- **Assay** : Assay is performed comparing the growth in test and standard sample.

APPLICATION

- Food Industry
- Pharmaceuticals
- Clinical Diagnostic

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