Important Question Of

Pharmaceutical Microbiology [Bp504Tp]

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AND

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Question.

1. Classification of Microscopy & TEM in Detail.
2. What is disinfectant & classify it?
3. How the bacteriostatic & bactericidal Action are evaluated & describe it.
4. Short note on preservation using the Anti-Microbial agent
5. What is spoilage write down the types of it and factor affecting the spoilage.
6. Write down the general Procedure for Cell Culture.
7. Give details on designing of Aseptic area with layout of Aseptic area.
8. Write a detail note on laminar flow equipment.
9. Write a detail note on Heppa filter.
10. Write a note on Methods of preservation of Contamination.
11. Scope of Microbiology & Application

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12. Classification of replication of fungal.

13. difference between SEM & TEM.
And
Prokaryotic and eukaryotic cell.

14. Morphological classification of bacterial with example and draw the stemetic diagram of bacterial cell.

15. Isolation & prevention of bacterial culture.

16. Enumarate the condition required for the growth of Micro organism draw typical bacterial growth curve.

17. enlist factor affecting growth of Microbes.

classify organism according to oxygen requirment temperature & nutritional requirement.

18. Write a note on Mechanical sterilization with detail.

19. draw the neat & labeled diagram of Hot air oven.

20. draw the neat & labeled diagram of Auto clave.

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Q.1* Types of Microscope :-

- Light Microscope
  - (1) Bright-field.
  - (2) Dark-field.
  - (3) phase-Contrast.
  - (4) fluorescence.
- electron Microscope.
  - (1) Transmission electron Microscope [TEM]
  - (2) Scanning electron Microscope [SEM]

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b⇒ There have two type of electron Microscope:-

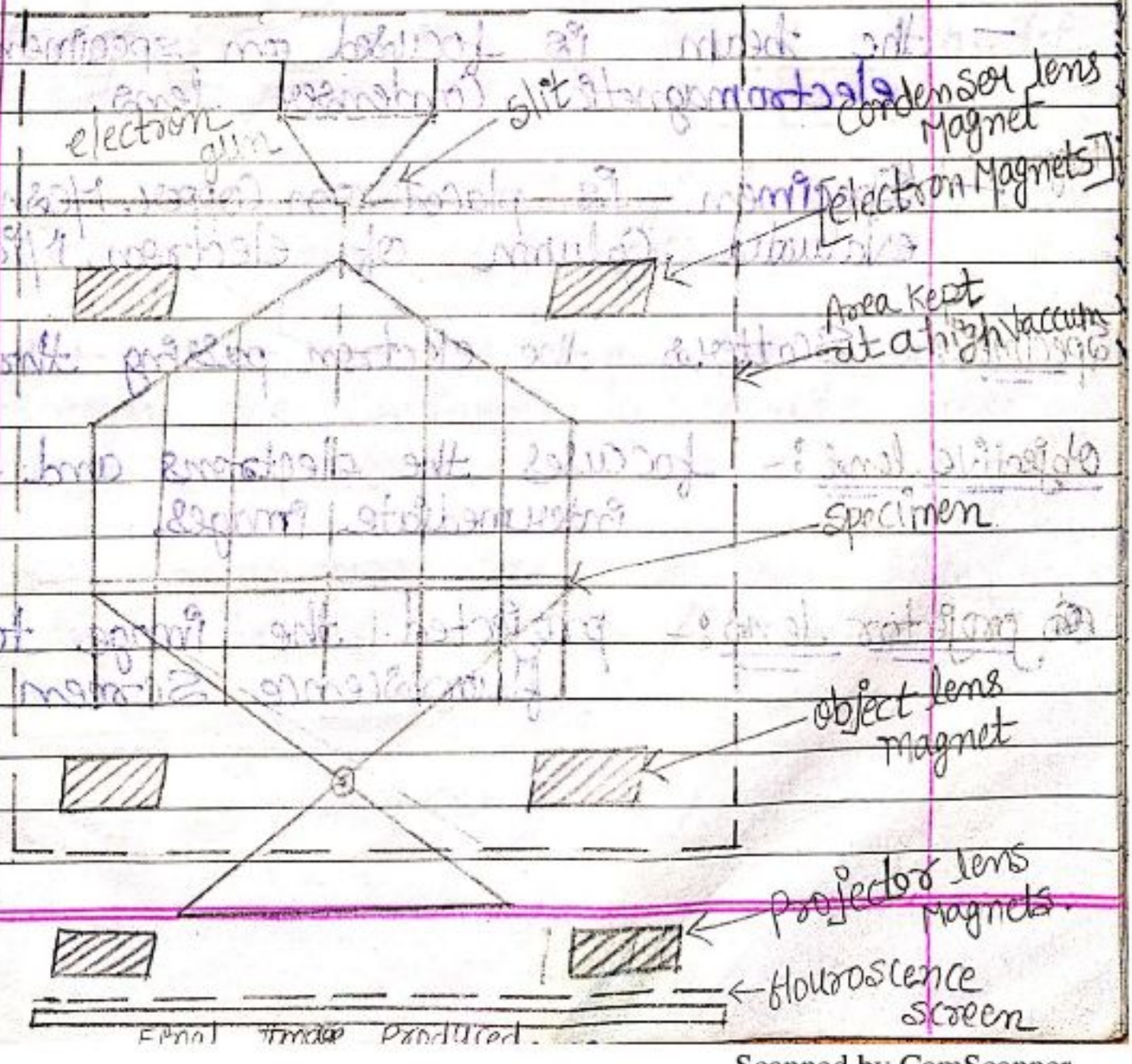
TEM

SEM.

Transmission electron Microscope.

Scanning electron Microscope.

(1) Transmission electron Microscope [TEM]:-



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Date
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ans:- A beam of electron and projected from an electron gun and passed through series of electromagnetic lenses.

- In TEM, electron beam is substituted for light source.

- Series of electromagnets are used instead of glass lens.

- electron source is hot tungsten filament in the electron gun.

- electrons are drawn from filament & accelerated as a fine electron beam.

- the beam is focused on specimen with electromagnetic condenser lens.

- Specimen is placed on copper mesh within evaluated column of electron microscope.

Specimen:- Scatters the electron passing through it.

Objective lens:- focuses the electrons and form intermediate images.

projector lens:- projected the image to fluorescence screen.

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→ Highly electron denser area scatters large amount of.

↓

allows less number of electron to pass through it.

↓

dark appearance Compard to

↓

less denser area.

Application: TEM :- higher resaluction.

- Electron Microscopy, air is removed & vaccum is created.
- In TEM, used as biological specimens are stained with heavy metal stains.
- Uses the electron beam, that passes through the specimen to visualize small images.
- use to observe small specimen like, tissue section. e.g :- Ebola Virus.

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Q2.

Definition -

"Disinfection is the process of destruction or removal of microorganisms and reducing them to a level not harmful to health."

→ If the object is inanimate (lifeless), such as working areas, dishes, benches etc., the chemical agent is known as a 'Disinfectant.'

→ Disinfectants are usually bacteriocidal but occasionally they may be bacteriastatic.

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Classification of Disinfectant

1) On the Basis of Chemical Structure

2) On the basis of Mode of action

1] On the basis of Chemical Structure

(i) Acid and alkalies -

→ conc^{n} of H^+ ions & OH^- ions responsible for anti-microbial activity.

ex- Benzoic acid and salicylic acid

(ii) Halogens -

Chlorine, Iodine, bromine and fluorine in the free state acts as Cresmicidal.

ex- Cl, F, Br, I

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(iii) Heavy metals-

→ Heavy metals and their compounds act anti-microbially by combining with cellular proteins.

Ex- Mercury, Silver & Copper.

(iv) Phenols-

→ Phenols and their derivatives have germicidal property and also acts as anti-Septics by inactivation of enzymes.

ex- O-cresol, m-cresol & p-cresol

(v) Alcohols-

→ Alcohols acts as bacteriocidal and fungicidal by protein denaturation.

ex- Ethanol, IPA (Iso-propyl alcohol)

(vi) Aldehydes-

→ Aldehydes have fungicidal, bacteriocidal, sporicidal & tuberculocidal action.

ex- Benzaldehyde & Formaldehyde

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(vii) Quaternary ammonium compounds-

→ They kill gram positive & gram negative bacteria & also some protozoa.

ex- Benzalkonium chloride.

(viii) Dyes-

→ Dyes are bacteriostatic in nature.

→ Basic dyes are more effective than acidic dyes.

ex- Acridine & Triphenylmethane

(ix) Detergents and soaps-

→ Cationic and Anionic gops of detergents & soaps are having bactericidal & fungicidal activity.

ex- Cetrimide, SLS (Sodium Lauryl sulphate)

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2] Basis of Mode of Action-

(i) Alteration of Microbial membrane-

→ Damage lipid complex in cell membrane, protein precipitation.

→ Dehydration occurs.

→ Denaturation of protein

→ Interfere with glycolysis and damage membrane

→ leakage of cell constituents.

Ex- Quarternary ammonium compounds, alcohol.

(ii) Protein denaturation-

→ Disruption of cell wall

→ precipitation of cellular protein

→ Inactivation of enzymes

→ Leakage of amino-acid from cells

→ Physical damage to cell membrane.

ex- Phenol & Alcohol

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(iii) Oxidation of Sulphydryl groups of enzymes-

→ Penetration into the cell wall of micro-organism and thereby causing disruption of protein and nucleic acid structure.

→ Loss of intracellular components

→ Chlorination of amino acids

→ Inhibition of protein synthesis

→ Oxidation of respiratory components

→ Decrease in ATP production

→ Decrease in DNA synthesis.

→ Damage to lipid, protein & DNA.

ex- Halogens (Cl, I) & Hydrogen peroxide

(iv) Alkylation of $-NH_2$, $-COOH$, $-SH$, $-OH$ grps of pyrine bases.-

→ The alkylation of $-NH_2$ & $-SH$ group of protein and ring nitrogen atom of

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purine bases, thereby damages nucleic acids.

→ Alkylation of $-NH_2$, $-COOH$, $-SH$ & $-OH$ group results in altered RNA, DNA and protein synthesis.

→ Alkylation of proteins leads to inactivation of enzymes

Ex- Ethylene Oxide, Formaldehyde & gluteraldehyde.

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Q3. Evaluation of Disinfectant

* 5 Method :-

1) Tube dilution & agar plate Method.
2) Filter paper & Cup plate Method.
3) Ditch - plate Method.
4) phenol Co-efficient Method.
5) Kelsey Sykes Method.

(1) Tube dilution & agar plate Method :-

In test tube or petri plate.

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Agar Media is filled.

↓

Disinfectant is incorporated.

↓

Micro-organism are inoculated.

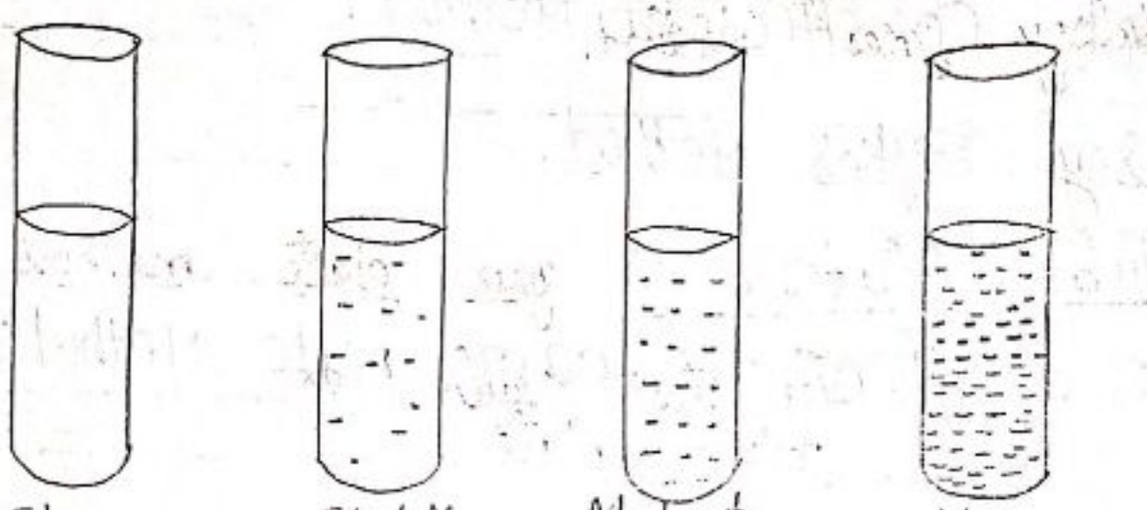
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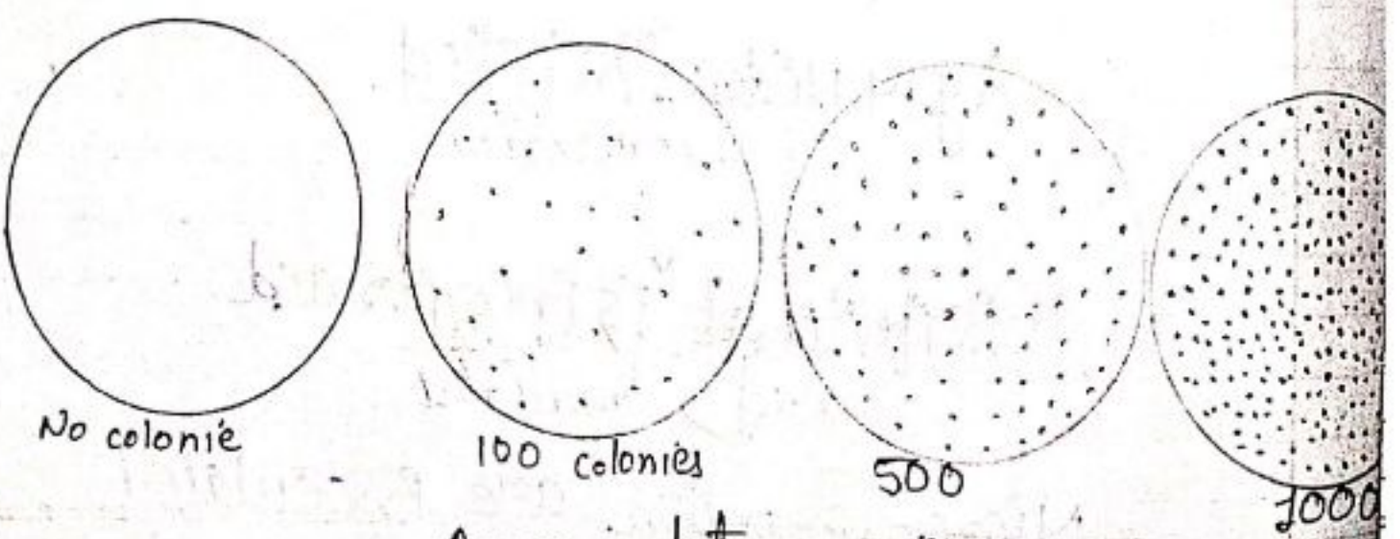
incubate for 24-48 hrs

↓

check the test tube in agar plate. ↓ turbidity in or colonies



Tube dilution method



Agar plate method

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2] Cup-plate method

First of all, in this method

↓

prepare the agar medium & sterilize it

↓

Directly incorporate the prepared bacterial culture when the medium is in liquid form.

↓

Directly add this media + culture into the petri plate & solidify.

↓

With the help of cork-borer scoop out the cavity from petri plate.

↓

Add disinfectant of different conc" in each cavities.

↓

Incubate it for 24-48 hrs.

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↓

The zone of inhibition of growth in each cavity is directly proportional to conc^{n} of disinfectant.

↓

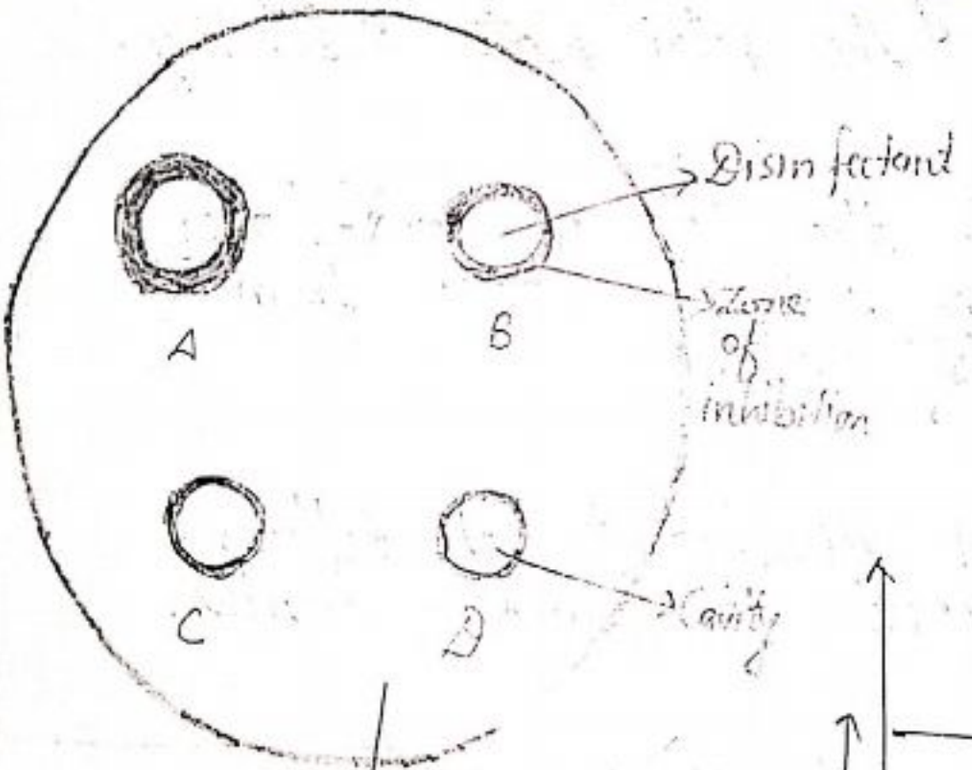
As the zone of inhibition ↑se means conc^{n} of disinfectant is more

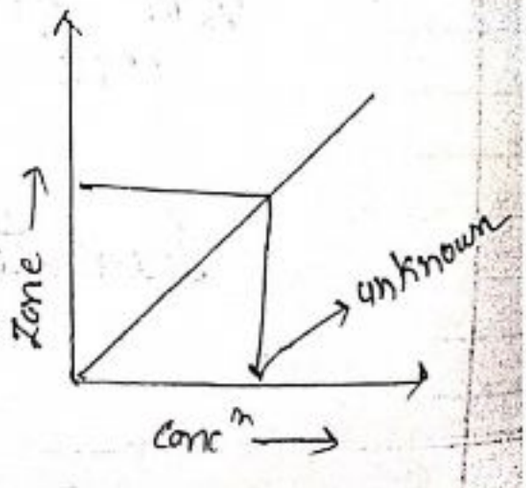
↓

By plotting the graph of Conc^{n} v/s zone of inhibition.

↓

we can find the conc^{n} of unknown disinfectant.





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3] Ditch - plate method

Prepare the agar media & sterilize it

↓

Directly incorporate the prepared bacterial culture when the medium is in liquid form

↓

Directly add this media + culture into the petri-plate & solidify

↓

With the help of cutter scoop out a ditch from petri-plate

↓

Add disinfectant in ditch

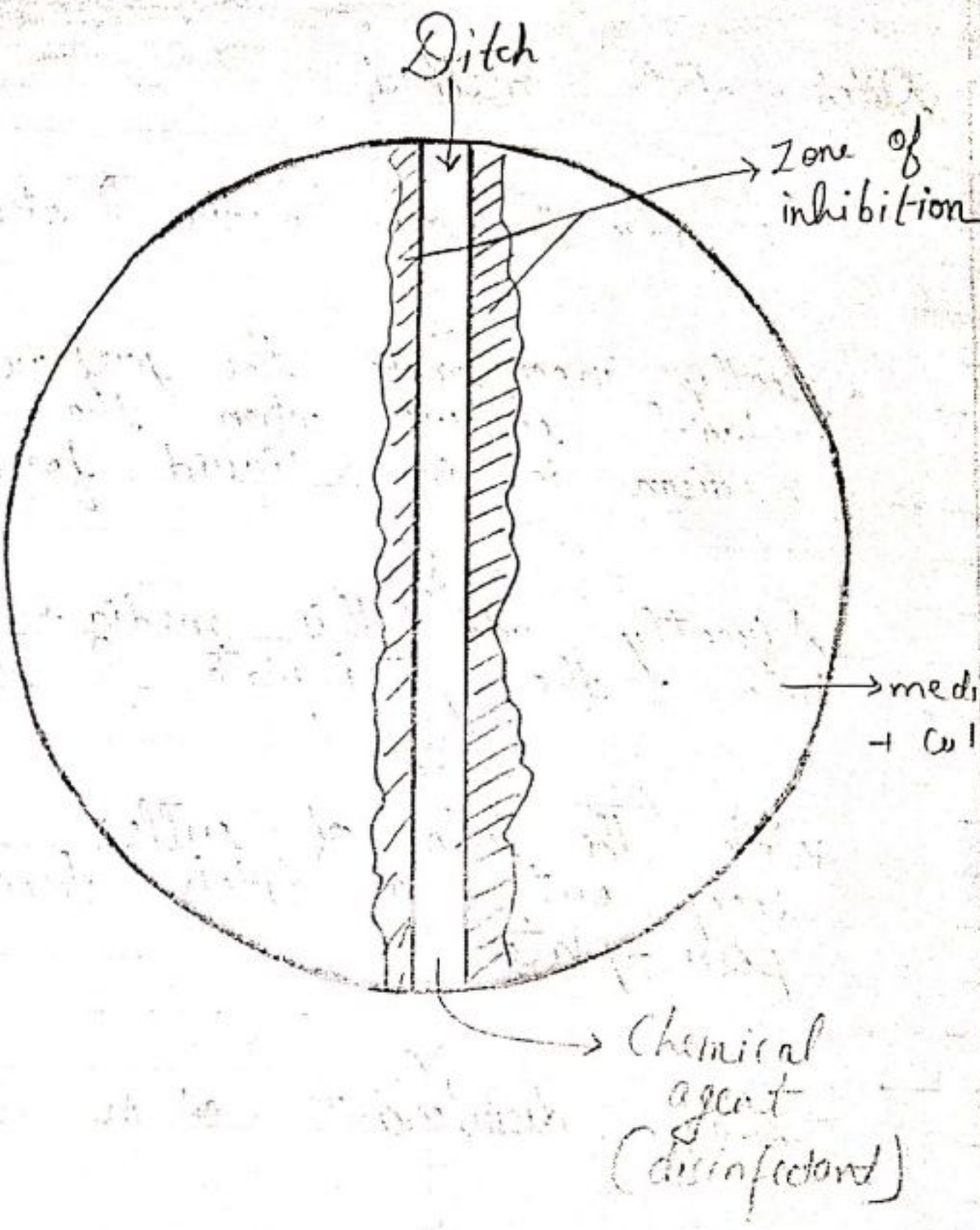
↓

Incubate it for 24-48 hrs

↓

The zone of inhibition of growth in each cavity is directly proportional to conc^{n} of disinfectant.

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Ditch - plate method

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4] Phenol-Co-efficient Method

→ In the phenol coefficient method a test chemical is rated for its microbial property with reference to phenol under identical conditions.

→ In this method similar quantities of micro-organisms are added to rising dilution of phenol and of the disinfectant to be tested.

The phenol co-efficient test includes-

(i) RW Test

(ii) CM Test

Rideal-Walker test

(i) RW Test [Rideal-Walker Test]-

- RW Test uses Salmonella Typhi as the sensitive micro-organism.
- Different dilutions of the test disinfectant and phenol are prepared and 5 mL of each dilution is inoculated with 0.5 mL of the 24 hr broth culture of organisms.

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→ All tubes are placed in a 17.5°C water bath.

→ Sub-culture of each mixture are taken and transferred to 5 mL sterile broth after 2.5, 5, 7.5 and 10 min.

→ Incubate at 37°C for 48 to 72 hrs and calculate RW co-efficient.

Disinfectant	Dilution	Time interval (min)			
		2.5	5	7.5	10
Test Disinfectant	1:1000	+	−	−	−
	1:2000	+	+	−	−
	1:3000	+	+	+	−
	1:4000	+	+	+	+
Phenol	1:80	+	−	−	−
	1:100	+	+	−	−
	1:120	+	+	+	−
	1:140	+	+	+	+

R.W. Coefficient = $\frac{\text{Dilution of disinfectant killing in 7.5 but not in 5 min}}{\text{Dilution of Phenol killing in 7.5 but not in 5 min}}$

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→ If RW co-efficient = 1, it means the test disinfectant has same effectiveness as Phenol.

→ If less than 1, it is less effective than Phenol.

→ If more than 1, it is more effective than phenol.

(ii) CM Test [Chick-Martin Test] Described

→ It is similar to RW Test.

→ Potency is determined in presence of organic matter, by using yeast suspension as diluents for the test disinfectant.

→ In this test, Dilution of the test disinfectant and phenol with water are inoculated with 0.2 ml of 24 hr old culture of microbes.

→ Mix and allow to keep at 30°C

→ Sub-culture are taken from each mixture and transferred to nutrient broth after interval of 30 minutes.

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→ These test-tubes are kept at 37°C for 48-72 hrs & CM co-efficient is calculated

$$\text{CM co-efficient} = \frac{\text{Mean conc}^{n} \text{ of phenol showing no growth after 30 min}}{\text{Mean conc}^{n} \text{ of Test disinfectant showing no growth after 30 min}}$$

→ Advantages of Phenol co-efficient method-

→ They are inexpensive and can be performed quickly.

→ Can give Reproducible results

→ They are valuable to eliminate useless products and supply standards for crude preparations.

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Disadvantages of Phenol Co-efficient method.

→ The microbes (Salmonella typhi) can not be killed by all type of disinfectant.

→ It compares the activity of disinfectant at only one conc" with a fixed death time & temperature.

→ If Organic matter is present, error is produced.

→ Sampling errors are large.

5] Kelsey - Sykes Method

Bacteria used -

Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa.

Time (min)	Boredure
0	Inoculate 3 ml of disinfectant dilution with 1ml of bacterial suspension in broth, yeast & shake.

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8 min	Collect sample from above reaction mixture with a pipette & Transfer 1 drop to each 5 tubes
10 min	To reaction mixture, prepared at start (time 0), add a second 1 mL of bacterial suspension.
18 min	Collect sample from above reaction mixture with a pipette & Transfer 1 drop to each 5 tubes
20 min	To reaction mixture, prepared at start (time 0), add a third 1 mL of bacterial suspension
28 min	Collect sample from above reaction mixture & do as of 18 min case.

Results - A disinfectant is satisfactory if..

(a) no growth occurs in 2 or more of the 5 tubes of the 18 min. sample

(b) there are not more than 5 colonies from the 5 drops on agar plate.

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Q4e:- Write down the general procedure for cell culture.

⇒ The cell culture method are either applide to ⟶ Free-living organism or to cell removed from multicellular tissues.

- cells can be cultured for a prolonged time period if they split regularly.

↓

After that growth medium replaced and cells are diluted.

- For culturing cells efficiently,

→ the environment in which they found themselves should be maintained before they are transplanted to the artificial environment.

→ This extra cellular fluid is recrected using Ham's tissue culture Medium. (commonly for mammalian cells)

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- Some cells survive without attaching to a surface, while others require special surface.

- There are also cells like,

  - yeast and
  - some bacteria types

  ↓

  which survive under either condition and exhibit diff. phenotypes depending upon wether or not they are attached to a surface.

- Most of cells derived from tissues

  ↓

  Nutrients are provided by liquid broth

  ↓

  that washes the cells attached to a surface

- Some cells, in this require an "air- liquid interface" for their growth the cells

  ↓

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In this case, are grown on a mat of organic material floating on the surface of a nutrient broth and acting like a wet sponge to feed the cells from underneath while the tops are exposed to the air.

- For Bacteria and yeast;

→ Small quantities of cells are grown on a solid support enriched with embedded in its STIFF nutritious Jellow, while large scale cultures are grown with the cells suspended in a nutrient broth.

- A population of cells derived from a single parental cell is called a clone,

↓

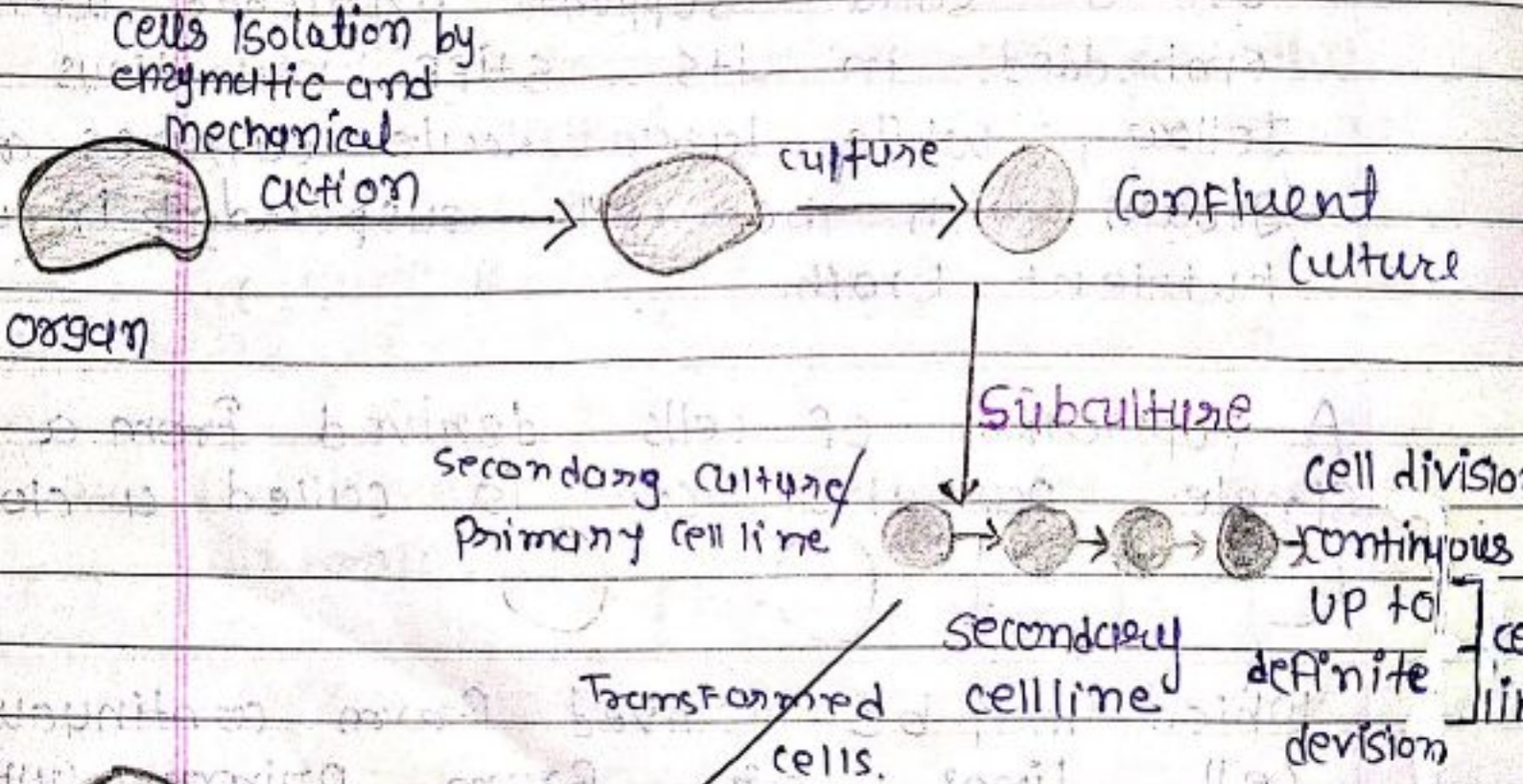
which may be derived from continuous cell lines or from primary cultures

- Clonning within a cell population reduces the degree of genetic and phenotypic Variation.

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⇒ Initiation of cell culture can be discussed under the following heads.

1. Prepⁿ and Sterilization of Substrate & culture vessel,
2. prepn and sterilization of Medium.
3. Isolation of cells and tissues.
4. Disaggregation of tissues.
5. Primary cell culture
6. Sub-culture
7. Clonning of cells lines
8. Contamination in cell culture.



Animal cell culture

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Que:- Short note on preservation using the anti-microbial agents.

- Antimicrobial preservatives are added to non-sterile dosage forms to protect them from microbial growth or from micro-organisms that are introduced inadvertently during or after the manufacturing process.

- Antimicrobial preservatives should be added if sterile article are packaged in multiple-dose container, thus they inhibit the growth of micro-organisms. introduced from repeated withdrawl of individual doses.

- preservatives are natural or synthetic chemicals added to prevent decomposition by microbial growth or by undesirable chemical changes in various pdt like foods, pharmaceuticals, paints, biological samples, wood etc,

- Thus, preservatives are added to various foods and pharmaceuticals in order to enhance their shelf life

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- chemical preservatives do not totally keep products free from getting spoiled by microbes, rather they slow down the spoiling process.

- During the processing of natural food, it is crushed, heated and added with the chemicals that may destroy all the nutritional value of the food

- often, Additives are added to the pdt to put back some vitamins and nutrients that were lost when the food pdt were processed.

- preservatives are added to keep the food fresh and from spoiling.

- Some commonly used natural preservative are vinegar, salt, and sugar.

- Most of the synthetic food and cosmetic preservatives are considered safe but some of them were found to be carcinogenic and toxic, and therefore have limited uses.

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- Generally, all synthetic chemical additives and preservatives may be avoided bcz of them are not tested properly.

- The food colouring chemicals have harmful effects, therefore use of food colourings made from synthetic chemicals should be avoided.

- The efficiency of antimicrobial preservatives depends on their ability to kill live cells., thus their toxicity avoidable.

- Many plants and compounds of Natural origin have the power to destroy bacteria, yeast and fungi.

- However, in Many cases these substances produce toxic effect on Humans.

For example :- citrus or grapefruit seed extracts have natural antimicrobial properties and some their constituent are responsible for the life - threatening hormonal imbalances.

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→ An antimicrobial agent should posses the following Ideal characteristics.

① It should be efficient against many species of micro-organism.
② It should possess high water solubility or easy dissolution in commonly used cosmetic ~~&~~ ingredients.
③ It should be safe and cause no irritation.
④ It should be neutral with no effect on product pH
⑤ It should not have any effect on product efficiency.
⑥ It should not have any adverse effect on product appearance.
⑦ It should be stable over wide range of temperature and pH
⑧ It should be available readily and its supply should be stable
⑨ It should be economical.

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★ Anti-microbial Agents Currently used in pharmaceutical products.

1. Single dose multiple dose injections.

Chloracresol (0.1%) Benzylalcohol (1%)
phenol (0.5%) Phenylmercuric nitrate
cresol (0.3%) acetate or borate
chlorbutol (0.5%) (0.002%)

2. Eye drops and contact lens solutions.

Chlorhexidine acetate, Thiomersal (0.1%)
or gluconate (0.1%), chlorbutol (0.5)
Benzalkonium chloride
(0.3%)

3. Oral liquids.

Methyl, ethyl and propyl
p-hydroxybenzoate (paraben) (0.3%)
Benzoic and Sorbic acid (0.3 - 05%)
Chloroform (0.25%)
Bronopol (0.5%)

4. Creams

paraben (0.3%), Bronopol (0.5%), Phenyl mercuric
Chlorocresol (0.1%) nitrate (0.01%)
cetyltrimethylammonium bromide (1.0%)

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Que:- What is spoilage ? Write down the types of it and factor affecting the spoilage

⇒ **Spoilage :-** The process of deterioration of pharmaceutical products by the contaminant microbes is known as microbial spoilage.

★ **Types of spoilage**

① Infection induced by contaminated pharmaceutical products :-
② Chemical and physicochemical Deterioration of pharmaceutical products
③ Ingredients subject to Microbial attack
④ Observable Effects of Microbial attack in pharmaceutical products :

① **Infection induced by contaminated p'ceutical pdts :-**

- Processing faults and contamination of injections during use produce localised infections or Septicaemia.
- Presence of a few microbial survivors in an injection may also induce infection.

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- Microbial growth occuring during storage of the pdt prior to its administration, results in a highly infective or toxic product.

Ex:-

(1) Severe eye infection have occured in patients using eye drops contaminated with pseudomonas aeruginosa.

(2) Ointments and creams if get contaminated causes dermatoses, burns, respiratory infections, and other diseases.

(3) Ineffectively sterilised and contaminated wash-out solution cause urinary tract infection.

(4) Oral Medication have a variety of micro-organisms, few of which are infective.

- tablets or capsule of yeast, thyroid, pancreatin and carmine are contaminated with Salmonella Species., causing infection.

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② Chemical and physicochemical Deterioration of Pharmaceutical products:

→ The degradation frequency of Micro-Organism is such that all organic matter are subjected to chemical modification under conditions milder than those for reaction performed by non-biological processes

- Biodegradable Material accumulating in the environment is the result of localised adverse condition or the absence of particular micro-organism

Ex:- lignins accumulate as peat and persistence of oil slicks in sea water.

- The degradation rate of ingredients depends on their chemical structure and physicochemical property.

③ Ingredients Subject to Microbial attack:

① Cationic Surfactant:- Gram negative bacteria grow extensively in this solution.

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(2) Anionic Surfactants :- Alkyli- metal and amine soaps of Fatty Acid are readily degraded in sewage but are normally stable in their slightly alkaline formulation.

(3) Non- Ionic Surfactant :- Ex :- Alkyl polyoxyethylene alcohol emulsifiers readily undergo metabolism by various micro- organism.

(4) Ampholytic Surfactant :- phosphatides and some betaine derivatives are readily undergo degradeition

(5) Organic polymer :- Agar is used as an inert support for culture Media bcz even if agar degrading Micro - organism are present agar readily Undergoes depolymerisation.

(6) Humectants :- Humectant are support to Microbial growth.

(7) Fats and oil :- Hydrophobia Material are attacked by microbes.

(8) sweeting, Flavouring and colouring agent This agent are used in pleutical prepn act as Substrates for microbial attack.

Follow the river and will reach the sea.

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(9) Potent therapeutic agent:-

=> Many drugs undergo gross degradation by various microbes.

(10) Preservative and disinfectant:-

- Most of organic preservative and disinfectant undergo metabolism by various bacteria and fungi.

(11) Observable effect of microbial attack in Pharmaceutical Pdt:-

- In a Pharmaceutical Pdt, microbial spoilage become visible if there is very high contaminant level.
- However, bacterial growth is observed on a surface moisture film or is indicated by the production of odoriferous product.
- microbial spoilage results in unpleasant taste and odour.
- Ex:- The o/w (oil in water emulsion) and creams serve as rich substrates for microbial attack.

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* Factors affecting the Microbial spoilage of pharmaceutical products

① Size of Inoculum :-

- Low levels of contaminant may be present in Pdt, it cause low rates deterioration.
- High level micro-organism
  ↓
  cause High (appreciable) microbial degradation.
- However, inoculums size alone is not always a reliable indicator of likely spoilage potential.

② Nutritional Factor :-

- Higher microbial spoilage of pharmaceutical Pdt generally requires appreciable growth of the contaminating micro-organism within the dosage forms.
- Most of the organism and ingredients act as potential carbon or nitrogen substrates for microbial growth.
- [illegible]

③ Moisture content :-

- The higher concentration of the solute in the formulation indicates that the water is low.

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- Most m.o grow best at high water activity.
- Hence, Formulation can be protected from microbes attack by lowering their water activity.
- moisture content of the air may be sufficient to initiate Fungal growth.

④ Temperature :-
- Spoilage of Pl ceutical could occur over the range of about -10 to 60°c
- Storage within specific, narrower temp. ranges ↓ will encourage particular group of Spoilage.

⑤ PH :-
- Extremes of pH prevent microbial attack
- Acidic condition favour fungal and yeast proliferation.
- Soap and mixture of magnesium hydroxide gel discourage microbial attack (8-9 PH)
- Antacid mixture, mouth washes and distilled water show growth of Pseudomonas species. and other gram negative bacteria.

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(6) Redox potential :-

- The cebility of microbes to grow in an environment is influenced by its oxin-Redn balance
- vaccum packaging of food stuffs or the inclusion of oxygen absorber in the package are the minimize oxygen level and Reduce microbial spoilage.

(7) Protective components :-

⇒ The preservative may also increase the resistance and durability of contaminant micro-organism

(8) Package Design :-

- The risk of microbial contamination of residual Pdt while using multi-dose containers by medical stuff or patient. is reduced by using unit dosage container.

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(9) Self Life of Product

- A product's expected life should be considered an important factor when the degree of microbial spoilage which may occur is being determined
- If a parentral solution containing a very low level of viable but damaged bacteria is stored for a prolonged period, the bacteria may undergo multiplication and release pyrogen., thus making product toxic

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Q.1 Give details on designing of Aseptic area with lay out of Aseptic area.

Ans:- The Sterile production unit and the general Manufacturing area within Hospital pharmacy or factor should be located Seperately.

- The Sterile Production unit Should be buffer.

ex:- Unauthorised Personal Should not gain acess to this unit.

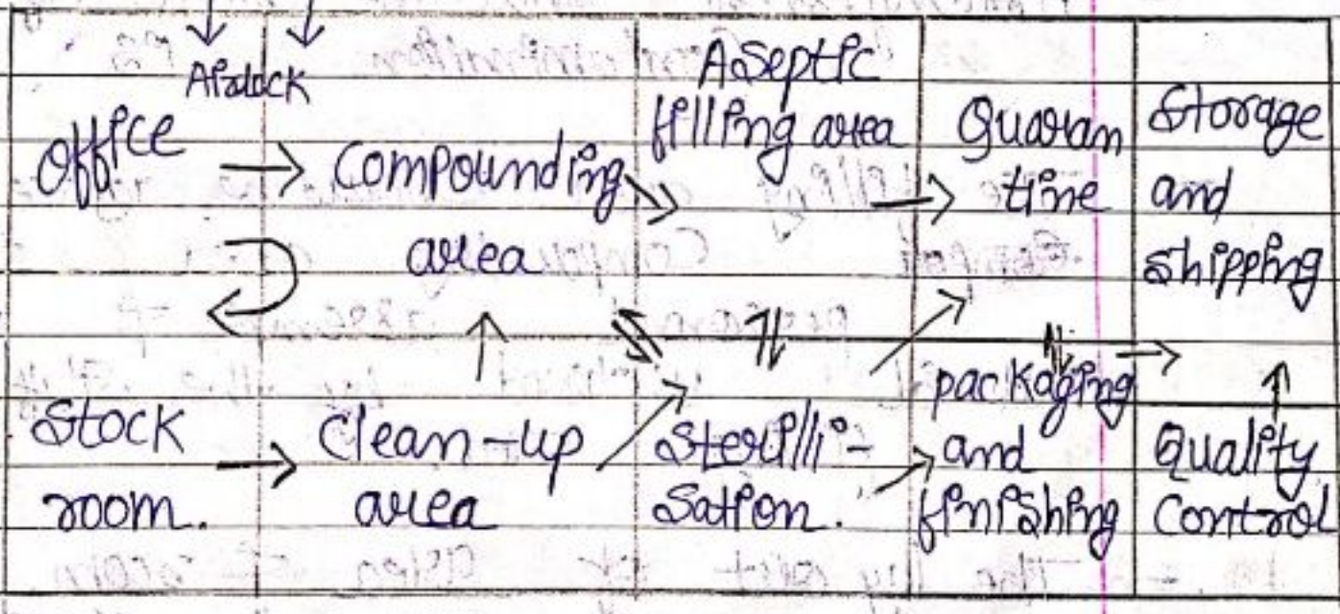


Figure :- Flow Diagram of Aseptic Area.

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- The Aseptic Unit is designed to each Stage of production Separately.
- The Unit Should Also ensure a Safe And Organised work flow So that the for personnel to move the clean room is minimised.
- The Unit is built & the equipment is Such a manner that the product remains protected from Contamination.
- The layout of Aseptic area Should be Such that cleaning can be done easily & dust accumulation can Also reduced.
- Arrangement that the risk of Cross Contamination is reduced.
- The filling area Should be adjacent to ~~Compo~~ Compounding area the personnel assemble & prepare Material utilised by the Staff in the filling area.
- The lay out of area & room for preparing terminally ~~Sterilized~~ Sterilized product is Show.

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* Design & Construction :-

- Only authorised personnel can gain acess to the clean and Aseptic filing area.
- The person enter the clean room by passing through the changing rooms where they put on and remove clean room uniform.
- A pass over bench extends cross the changing room to form a physical barrier for separating the different areas for changing by the persons.
- Special precaution are taken for preventing clean & Aseptic area from contaminated material are passed through airlock or Hatchway.
- Thus, steriliser & entry port are fitted with double sided doors, which are interlocked to prevent simultaneous opening of both the doors.

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* Surfacing Materials :-

- The floor, wall & ceiling surface should be smooth, impervious and unbroken.
  ↓
  To reduce the release & accumulation of contaminating particle & organism.

- Surface material should the effect of cleaning agent and disinfectants.

- Uncleanable recesses should be avoided within clean rooms to minimise the accumulation of contaminating particles

- Thus, the wall & floor junction should be covered.

- Minimum shelves, ledges, cupboards & equipment should be present.

- Non-opening & sealed windows should be present to prevent the entry of contaminants.

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Services :-

- Piped liquids and gases entering the clean rooms should be filtered.
- The position of pipes & ducts should be easily cleaned.
- Other fitting, like fuse boxes & switch panels should placed outside the clean rooms.
- Sinks and drains should not present in Aseptic areas.
- where Aseptic procedures are carried out within the clean room areas.
- They present in complete unit.
- Sinks & drains should designed, positioned & maintained that the risk of Microbial Contamination is reduced.
- they are fitted with easily cleanable traps, electrically heated disinfection devices.
- A limited ~~not~~ number of doors & ports, should ~~be~~ present.

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- The entry doors should self-closing to allow.

- Airlock doors, wall ports, through the wall, autoclave and dry heat steriliser should simultaneous opening of both the doors.

- All the doors should have alarm System should ring

↓

when more than one door are being opened.

- Light in clean room should fitted with ceiling so, the dust accumulation reduced.

- The equipment in clean room should be position that accumulation of particle & Microbial Contaminant does not occurs.

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Q2 Method of prevention of Contamination:

- The Steps by which Microbial Contamination of product can be prevent in Aseptic areas are discussed below.

(1) personnel :-

→ Acess to production areas by the unauthorised personnel should be restricted and only trained one should be Allowed to enter.

→ Adequate personnel hygiene Should be Maintained

→ The person should be given proper & regular training with respect to hygiene & that their activities do not hamper the product quality.

- personnel entering the Manufacturing area should wear protective clothing. (over-garments, Hair cover, beard or Moustache cover and overshoes)

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- Personnel entering the Manufacturing with naked Hands the exposed Products or any part of equipment in Contact with the Product.

(2) Facility Design :- This includes the following measures

→ In Aseptic rooms, the differential air Pressures should be ~~set to attain the defined~~ Higher than the adjacent controlled areas.

→ Air filtration & air change rates should be set to attain the defined clean ~~room~~ room class.

- Ambient temperature & humidity should not be very High

→ The areas of unequal risk are separated by UV air lock and door barriers.

→ All open containers processing should be performed within the isolator/RABS

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- HVAC air distribution components should be used for preventing the spreading of contaminants generated within the room.
- All rooms & surfaces should be maintained & monitored for viable and non-viable particulates and this facility should be re-certified on a semi-Annual basis.

⇒ facility & equipment design cannot eliminate microbial contamination completely, but good risk assessment and consequent hygienic design can reduce these risk several times.

forex:- (i) flow of man & material should be uniform.
(ii) equipment having smooth surfaces of appropriate material can facilitate effective cleaning.
(iii) closed material transfer systems.

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(3) Access to Areas :-

- Acess to production, packaging & storage areas by unauthorised personnel should be restricted.
- Personnel should gain Access to these areas only via changing rooms.
- Materials should be Accessed via specific routes (generally air locks).

(4) Building requirement :-

- Smooth, crack-free, & easily cleanable floors, walls, and ceilings should be used as they facilitate easy and effective cleaning.
- The designing of pipe work, Ventilation and light point should be such that creation of recesses which are not easily cleanable is avoided.

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- Sinks of stainless steel should be present within the production areas.

(5) Cleaning & disinfection :-

- Time to time cleanliness & disinfection of the areas should be done.

- Adequate hygiene should be Maintained in every Aspect of the drug product Manufacturing.

- Cleaning agents of proper grades should be used for minimising Health risks.

- The cleaning Agents should not be directly Applied on the product.

- The cleaning practices should be Validated to prove that process effectively controls Microbial contamination.

(6) Utilities :-

- Water of pharmaceutical grade, Microbiologically Controlled and Monitored should be used in the Manufacturing of Product.

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(ii) Clean & additive-free steam should be used for cleaning and sanitization of production production tools and equipment and to supply for autoclave and Humidification.

Q3 Short note on Laminar flow equipment.

And

Laminar Airflow Hood

Ans. A Laminar Airflow Hood (LAFH)/Laminar Aseptic Hood or a Workbench is a primary engineering Control device which provide the following services during aseptic Compounding :-

(1) Clean air to the Critical Sites. (Immediate Aseptic Compounding area).

(2) Constant flow of air out of the Work area to prevent the entry of room air.

(3) Outward flow of air from the hood that suspends and remove Contamination which have been introduced in the work area by personnel.

Ans. The LAFH works on the principle that twice filtered laminar layer of Aseptic air Continuously sweep the work area within the Hood to prevent the entry of Contaminated room air.

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* Types of LAFH :-

* A laminar air flow Hoods is of two types :-

(1) Horizontal Air flow Hoods :-

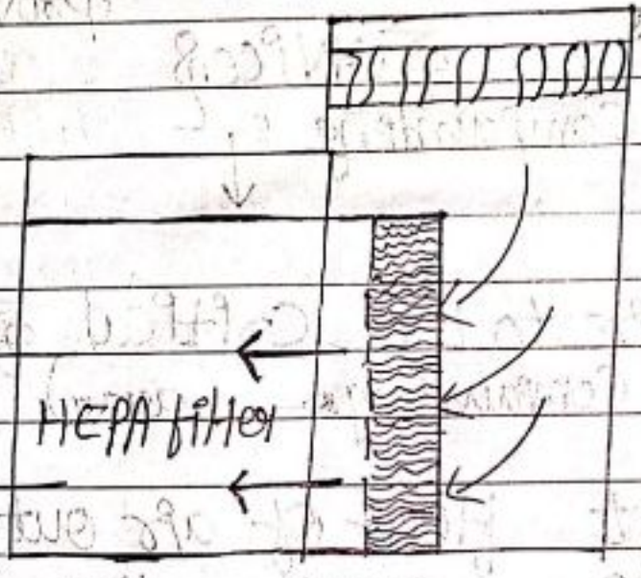


Figure :- Horizontal Laminar Air flow Hood.

- These Hoods sweep the filter air from back to the front of the Hood.
- An electrical blower draws the contaminated room air using a pre-filter (similar to a furnace filter), to remove gross contaminants. The pre-filter finely

→ The pre-filter timely cleanliness & replacement.

→ Airflow distribution the final filter (HEPA filter), present back portion of the hood's working area.

→ HEPA filter removes 99.97% of 0.3 u or larger sized particles, thus removing most of the 0.5 u or larger sized airbone microorganisms.

2) Vertical Airflow Hoods :- These hoods sweep the filtered air Vertically. ~~The air~~

→ The air passing through HEPA filter merges from the top and passes downward through working area. ~~It~~

→ Vertical airflow hoods work principle that air flow between the HEPA filter & Component used for CSPs preparation should not be interrupted.

→ Wind turbulence in the critical area increases & contaminants sterile work surface, thus contaminating the injection port needle or syrings.

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Figure :- Vertical laminar Airflow Hood

→ The materials within the compounding work area interrupt the pattern of air blowing from the HEPA filter.

* Uses :-

1) It has various applications, especially where an individual clean air environment is required for smaller items, e.g., particle sensitive electronic devices.

2) It is useful for special operations in laboratories.

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* Advantages :-

1) The Sterile area is spacious.

2) Since the Sterile area does not become hot, the plant materials can stay for a longer duration.

3) Bigger flasks having wide lids can be used.

* Limitations.

→ The airflow velocity can be overcome easily without any strict work practices & aseptic technique, thus an area of turbulence is created which can introduce room contaminants into the critical work area. A laminar

→ A laminar air flow hood should remain On for 24 hours.

→ It is turned off, it should be left On for atleast 30 minutes for its disinfection before use.

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Q4. Write a note on Hepa filter.

Or

A. High efficiency Particulate Air

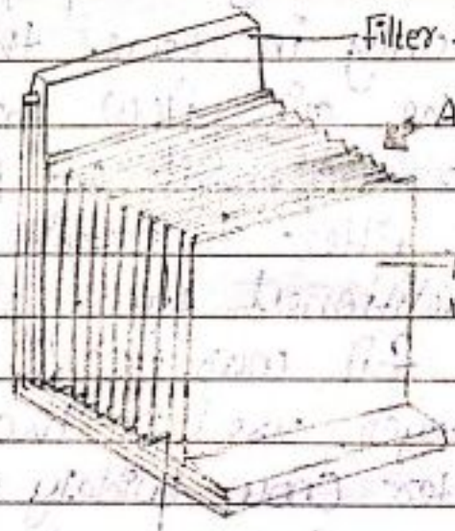


Figure :- Section through a Mini-pleat High-Efficiency Filter.

A High Efficiency particulate Air (HEPA) Filter is the most important part of a LAFA. The air within the room is taken into this filter and passed through a pre-filter which removes the gross contaminants (lint, dust, etc.). The air is then blown at a uniform velocity through the hood and HEPA filter in a unidirectional (laminar flow) manner over the critical sites (immediate aseptic compounding area). HEPA filter is a particulate filter which traps the airborne particles and microbes; but allows the gases to pass through.

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- HEPA filter should be fitted either at or near to the clean room inlet. A pre-filter is fitted upstream of the HEPA filter, thus extending the final filter's life. A fan is also fitted which pumps the air through the filter.

- The filter medium used in HEPA is made up of pleated fiberglass paper. parallelly arranged pleats not only increase the filet surface area but also the air flow through the filter. This parallel arrangement of filter medium also allows the filter to retain a compact volume. In the traditional type of HEPA A filters, aluminium foil was used as spacers, which are no longer used in the modern mini-pleat type of filter (now widely used). The mini-pleat filters have a shallower depth in construction than the traditional HEPA filter. The filter material is sealed to an aluminium frame within the filter. One side of the filter is protected with a coated mild steel mesh.

⇒ HEPA filters provide :

1) At high air flow rate,
2) A high particulate holding capacity, and
3) A low-pressure drop across the filter.

- HEPA filters remove large, medium, and smaller particles from the air by inertial impaction, direct interception, and by Brownian

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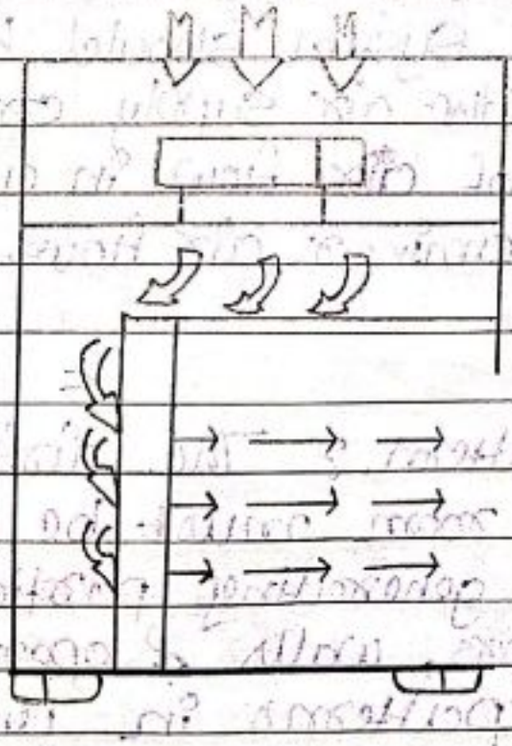
diffusion, respectively. The HEPA filters are least efficient in removing particles of above 0.3 um size. However, its efficiency of removing particles is effected by air velocity and filter packing. It removes largers and smaller particles with more efficiency.

→ It have two types

1) Vertical laminar airflow hood.

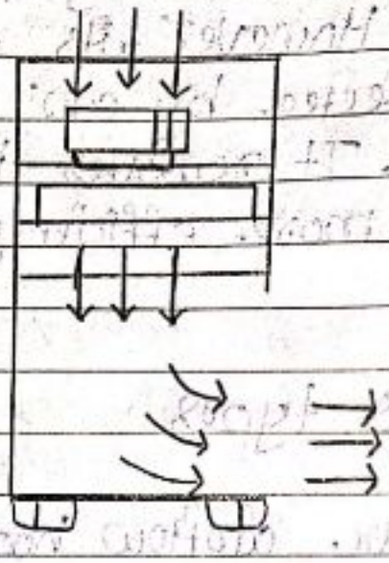
2) Cross-sectional of Horizontal laminar airflow hood.

(1.) Horizontal ~~Vertical~~ laminar airflow hood :-



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(2) ~~Horizontal~~ Vertical Laminar air flow. :-



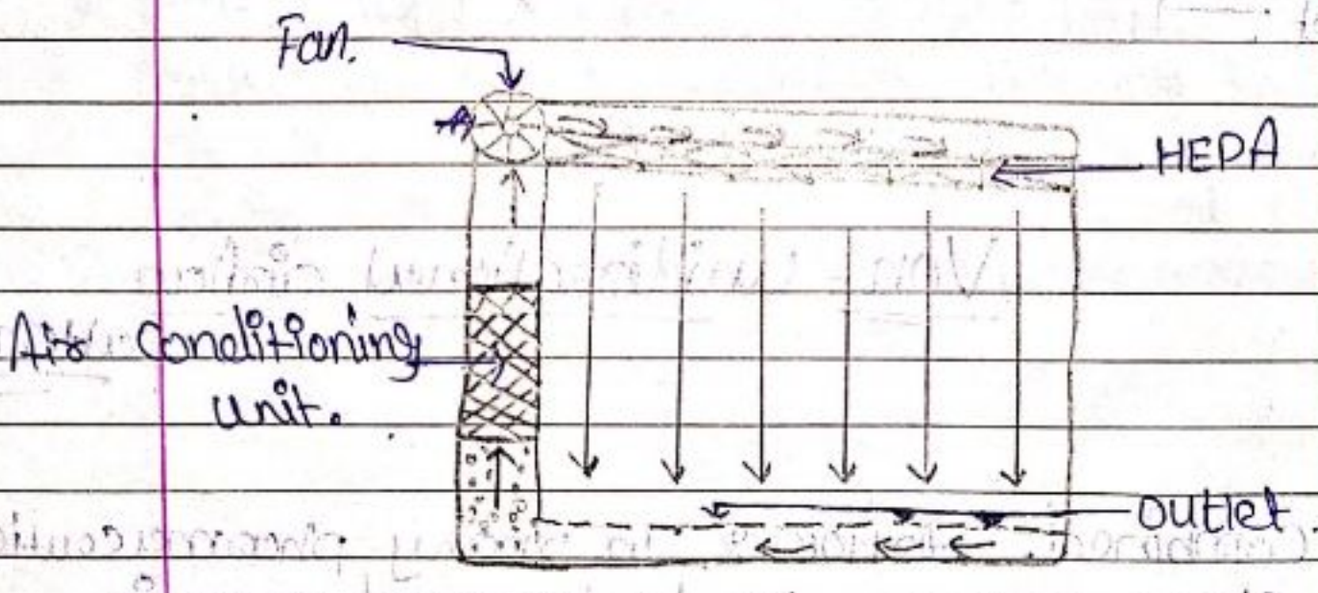
This is a ~~the~~ universally acceptable challenge test for HEPA filters. Dioctyphalate smoke is introduced at the supply plenum and photometer probe. Scan the surface of the filter for smoke particles. Dioctylphthealte is a volatile liquid ~~on~~ under pressure, it converts vapor or smoke a size range of 0.4 um Leakage filter the particles to and this detected by the photometer. An alarm system should be used to indicate failure in the air supply or filter blockage. Direction of air flow in a horizontal and vertical laminar air flows.

Airflow pattern :- The airflow pattern within the clean room must be carefully regulated to avoid generating particles from the clean room floors, walls & operators. The general airflow patterns in unidirectional airflow,

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non-unidirectional airflow and combination airflow.

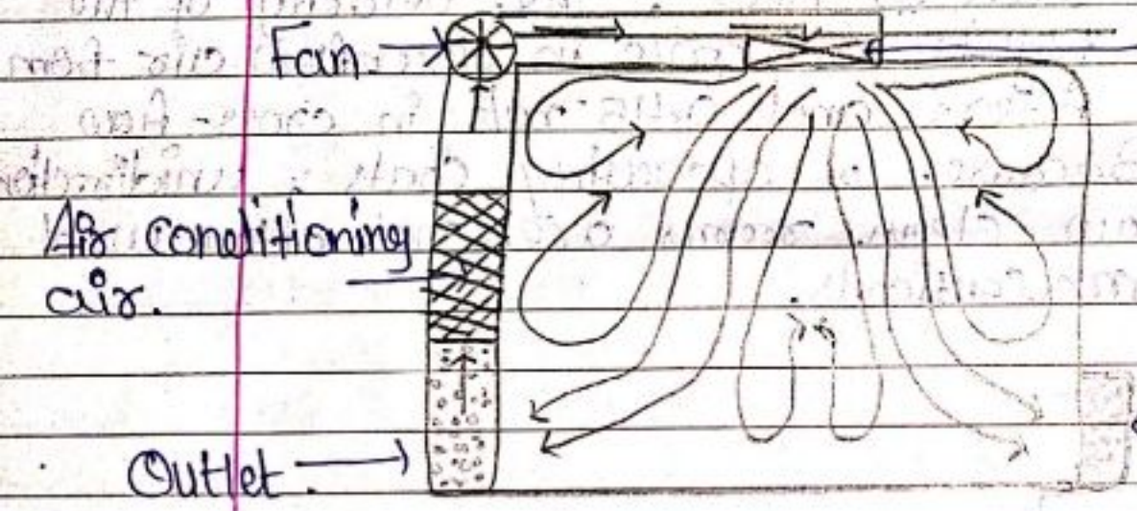
(1) **Unidirectional Airflow:-** The air within the room moves with uniform velocity along parallel flow lines. Air enters the room through a bank of filters and exits through a bank of outlets comprising the opposite wall or floor. The velocity of the air is highly 0.3 m/s in downflow air from ceiling filters and 0.45 m/s in cross-flow air. Because of operating costs, unidirectional airflow clean rooms are not often used in pharmaceuticals.



Unidirectional airflow pattern.

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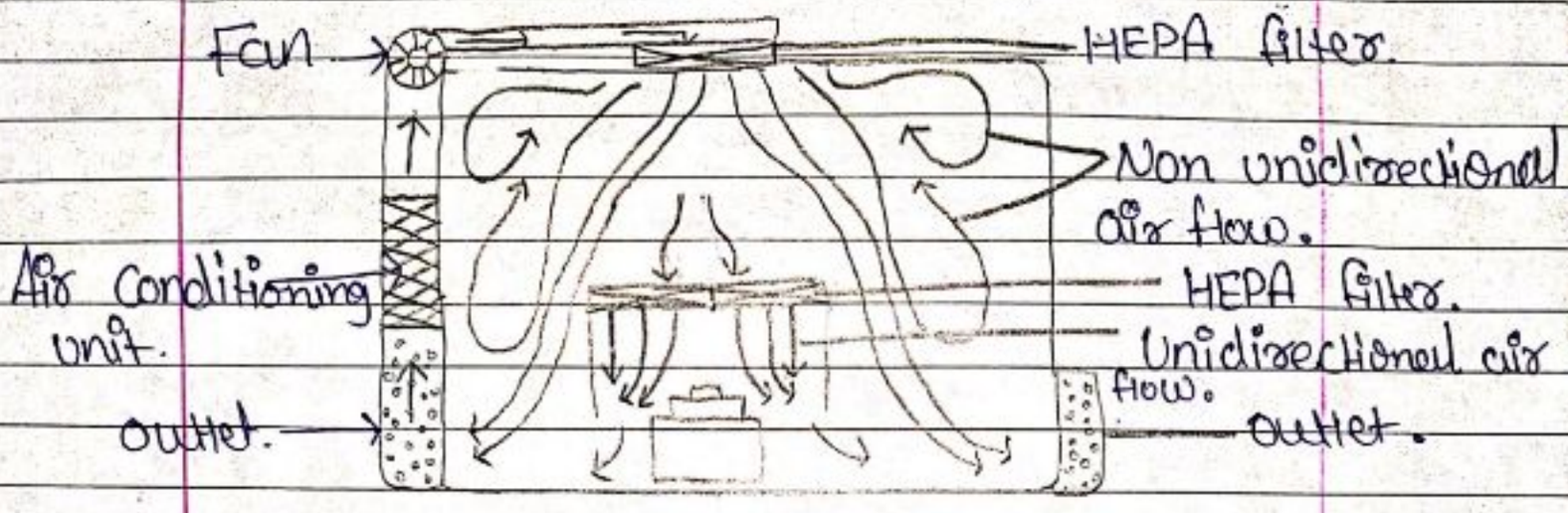
② Non-unidirectional airflow :- The air enters the clean rooms through filters and exits through outlet ducts, positioned low down on the wall or in the floor at sites remote from the air inlet. Conventional airflow is defined in terms of the numbers of air changes per hour (← 20 air change/hour).



Non-unidirectional airflow pattern.

③ Combined Airflow :- In many pharmaceutical clean rooms, the background area is ventilated by a non-unidirectional airflow and critical areas are supplied with unidirectional airflow. Various vertical unidirectional airflow system are used in combination clean rooms.

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Combined airflow pattern.

- The temperature and the humidity are adjusted to procedures within the clean room. A temperature of about 20 to 22°C with a relative humidity of about 35 to 50% are often preferred.

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9/11/.

Page 03.

Application or Scope of Microbiology in various field :-

(1) Pharmaceutical Microbiology :- It deals with the Manufacturing of life-saving drug termed as 'antibiotic'

ex:- penicillins, chloramphenicol, tetracycline, ciprofloxacam, streptomycine.

(2) Industrial Microbiology :- It is focuse on Making of ethanol, acetic acid, lactic acid, citric acid, glucose syrup, high fructose-syrup. It also include production of pharmaceuticles.

(3) Medical Microbiology :- It deals with the diagnostic protocol for identification of causative agent of various human disease or disorder.

(4) Beverages & Spirit Microbiology :-

ans.. It deal with the preparation wine & a variety of alcoholic beverages.

e.x:- wisky, brandy rum, gin.

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⑤ Fermented food Microbiology :- it deals with the preparation of cheeze, yoghurt.

⑥ Geochemical Microbiology :- it is help in the study of coal, Mineral deposite & gas formation.

⑦ Agricultural Microbiology :- help in the Maintainance of soil fertility.

⑧ Waste treatment Microbiology :- it deal with the treatment of domestic & industrial effluents or waste with the help of Micro-Organism.

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* Importance of Microbes in the pharmaceutical Industry :-

(1) Manufacture of antibiotics.

(2) Manufacture of steroids.

(3) Manufacture of therapeutic enzyme.

(4) Manufacture of recombinant DNA product.

(5) Used in production of vaccine.

(6) Sterilization Monitoring & validation procedure.

(7) Manufacture of Vitamin.

(1) Manufacture of antibiotics :-

- Various bacteria & fungi play imp role in production of antibiotics.

ex:- (a) penicillin ⟶ penicillin notatum.

(b) Streptomycin ⟶ Streptomyces griseus.

(c) Bacitracin ⟶ Bacillus subtilis.

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② Manufacturing of steroids:-

- Conversion of progesterone.

↓

11α - hydroxyprogesterone

↓ Mediated by.

{Rhizopus nigricans & Rhizopus arrhizus.}

- Conversion of Cortisone.

↓

Hydroxy Cortisone.

↓ Mediated by

{Curvularia lunata.}

- Testerone was produce from.

↓

dehydroepeandrosterone

↓ By.

{Corynebacterium species.}

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③ Manufacture of therapeutic enzyme :-

- Glucose oxidase → Aspergillus niger.
- Glycerol Kinase → Candida Mycoderma.

④ Manufacture of recombinant DNA :-

- Referon -A & Intron-A are produced with the aid of escherichia coli.
- Humulin is also from → Escherichia coli or E. coli.

⑤ Used in production of Vaccine :-

- live Vaccines → polio.
- tuberculosis → Bacillus Calmette-Guerin
- attenuated Varicella zoster virus.
  ↓
  chicken pox.

⑥ Sterilization Monitoring & Validation procedures

- Autoclave → Bacillus stearothermophilum.
- Dry heat → Bacillus subtilis var.
- Ionising radiation → Bacillus pumilus.

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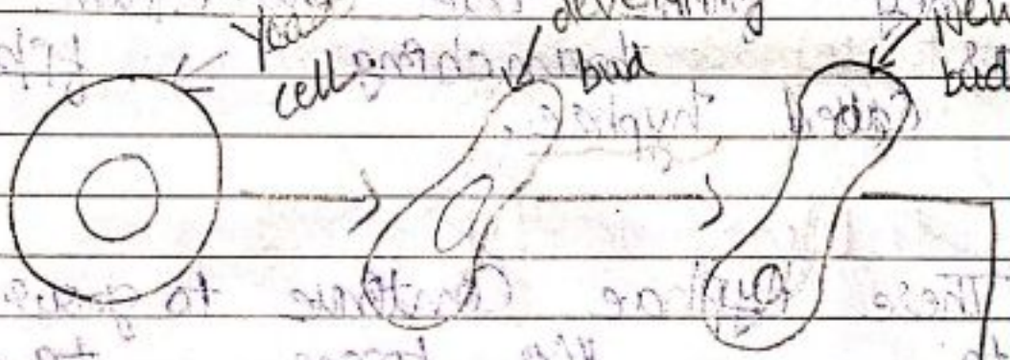
⑦ Manufacture of Vitamin :-

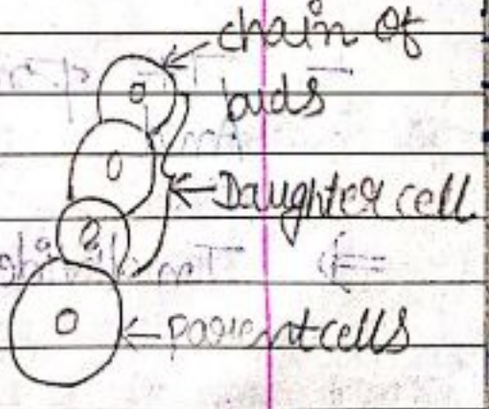
... Vitamin B_2 (Riboflavin) is produced in good yield by the Mould Eremothecium ashbyii & Ashbya gossypii grow on protein.

Q.12.* Classification of fungi :-

(1). Morphological classification :-

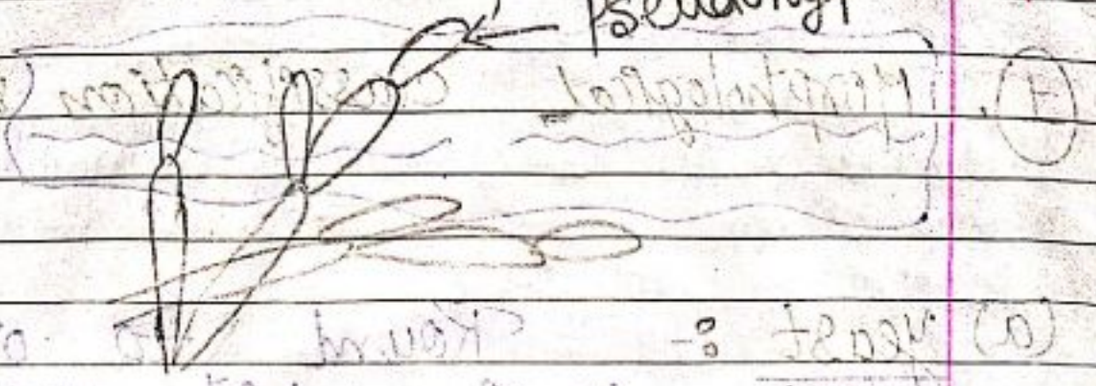
(a) Yeast :- Round to oval unicellular fungi, which mostly reproduce asexually.





(b) Yeast like fungi :- They grow partly as yeast and partly as chains of elongated budding All which are joined and to end forming pseudohyphae.

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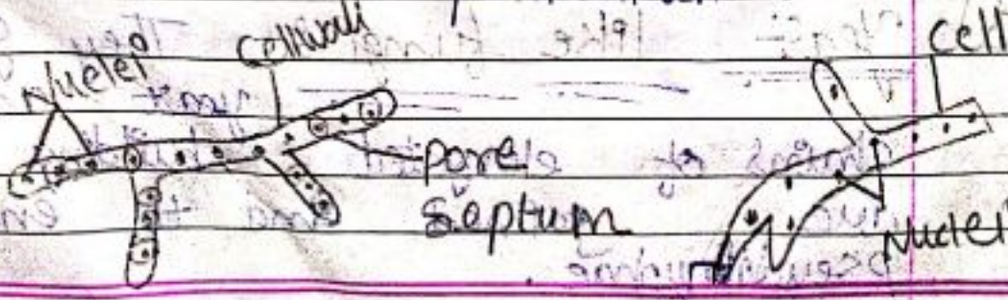
(C) Mould or Filamentous fungi :-

⇒ They form true mycelium and grow as branching filament called hyphae.

– These Hyphae continue to grow and branch to form tangled Mass of growth called Mycelium.

– It produce different type of Sexual And Asexual.

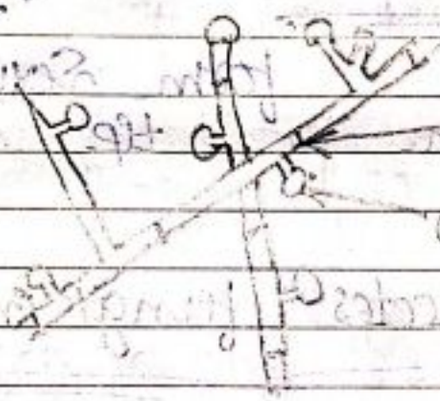
⇒ Individed into Dermatophyta
zygomycetes
Aspergillus
penicillium.



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(d) Dimorphic fungi :- This are those that exist as two different Morphological forms. fungi exist either as yeast or as moulds depending upon the growth conditions.

ex:- Histoplasma Capsulatum
Blastomyces dermatitides.
Coccidioides immitis.



(2) Taxonomical classification :- [basis of formation of Sexual Spores]

(a) Zygomycetes :- These include lower fungi with non-separate hypha.

↓ Asexual spore Sporangio spores.

↓ Sexual Spore oospores and zygospores.

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(b) Ascomycetes :- They form Septate hypha and form Sexual Spore called as Cospores.

(c) Basidiomycetes :- They form Septate hypha and form Sexual Spore called as

(C) Basidiomycetes :- They Also posses Septate hypha and form Sexual Spore outside on tip. [basidospore]

(d) Deuteromycetes [fungi imperfect] :- Also posses Septate hypha but doesn't produced Sexual Spores.

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Date
Page 06

Reproduction Of fungi :-

Ans:- fungi reproduce Sexually And Asexually.

(1). Sexual Reproduction :-

Ans:- In Sexual reproduction there occurs fusion of two different cells [Male & Female] called plasmogamy followed by fusion of two nuclei called "Karyogamy."

- As a result zygote is developed with formation of Sexual Spores which May be ooSpores, Zygospores, AScospores And basidiospores.

(2). ASexual Reproduction :-

Ans:- fungi which are Medically Important reproduce by this Method.

(a) Vegatative Spores :-

(i) Blastospores :- formed by budding process from Parent Cells as in yeast.

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(ii) **Arthrospores** :- formed by fragmentation of ends of Hypha which result in production of rectangular spores.

(iii) **Chlamydospores** :- These are thick walled resting spores which develop from hyphae after long dormacy.

(b) **Aerial Spores** :-

(i) **Conidiospores or Conidia** :- These are the spores which are borne externally on side of tips of special Hypae.

- When spores are small in size (singly).
  - ↓
  - Called as **Microconidia**

- When spores are large in size (multi cellular)
  - ↓
  - Called as **Macroconidia**.

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(ii) Phialospores :- They are Modified Conidia borne at tip of a flaske shaped specialize Conidia called phialide.

(iii) Sporangiospores :- These are Spores formed within A Sac on the ends of Specialized Hyphae.

* Cultivation of Fungi :-

⇒ Sabouraud's dextrose agar [SDA] [Vaginal Culture] or SDA with Antibiotics. Has been routinely used for primary isolation of Mycotic agents from clinical Specimen.

⇒ for fastidious [Special nutrients are Includes] pathogenic Special potato flake Agar [PFA] Inhibitory mold agar [IMA].

⇒ Combination of SDA + SA [BHI] Brain Heart Infusion.
↓
is recommanded.

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Q.* Difference between transmission electron Microscopy and Scanning electron Microscopy.

Q.13

Transmission electon Microscopy [TEM]	Scanning electon Microscopy [SEM].
- It has Maximum Magnification power about 1,00,000X.	- It has Maximum Magnification power of about 10,000X.
- It has resolution of 0.5nm.	- It has resolution of 10nm.
- this instrument is distinct from scanning electron Microscope because the electron are given very high energies with the help of electromagnets.	- electrons are not gives high energies.
- imaging is the result of electrons the specimen, either to fluorescence on a imaging plate or expose photographic film.	- Scanning produce the image from the electrons emitted by the object surface rather than the electrons passing through the specimen.
- Section of specimen are viewed under very high	- Scans and Magnifies external surface of

Scanned by CamScanner

	Magnification, finest detailed structure of cells and viruses is shown, used only on preserved Material.	Specimen.
✓ —	Does not produce three dimensional images.	— produces striking three dimensional image.

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* Difference :-

	Prokaryotic Cell.	Eukaryotic Cell.
①	lack of nucleus.	① having well defined nucleus.
②	number of chromosome one.	② More than one.
③	Cell membrane covered by rigid cell well.	③ Cell membrane covered by flexible plasma membrane.
④	Size of cell is 1-10 um.	④ Size 1 to 100 um.
⑤	Cell division exist by fission.	⑤ Cell divided by Mitosis process.
⑥	organelles & cytoskeleton absent.	⑥ organelles & cytoskeleton is present.
⑦	Cell wall have Complex chemical Composition.	⑦ Simple chemical Composition.
⑧	Ribosome Smaller than eukaryotes.	⑧ larger than prokaryotes.
⑨	e.g:- Bacteria, Rickettsiae.	⑨ e.g:- plant, animal.
⑩	lysosome - absent. - Mito chondria - absent.	⑩ present in human.
⑪	Golgi apparatus - present. - chloroplast - absent.	⑪. present in plant.

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* Morphological classification :-

⇒ Bacteria show great variation in their shapes :-

1. **Cocci** [Greek Kokkos - grain or seed] :-
   - These are are spherical or oval cells

(a) Micrococci :- Coccal that occur singly.

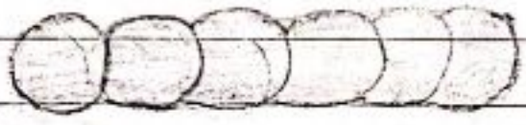


[Micrococcus]

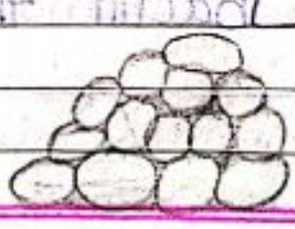
(b) Diplococcal :- Cocci that occur in pairs. [diplo meaning pair]



(c) Streptococcal :- Coccal that occur in chain. [strepto meaning chain]



(d) Staphylococcal :- Cocci that occur in bunches. [Staphylo meaning bunch]

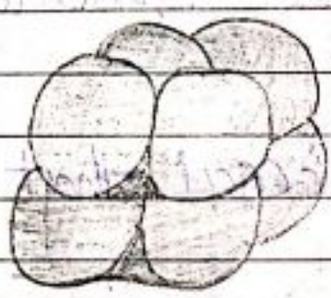


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(e) Tetrad - cocci :- that occur in groups of four [Tetra meaning four]



(f) Sarcinae (Octad) - cocci :- that occur in group of eight [octa meaning eight].



2. Bacilli [Latin bacillus - stick] :-

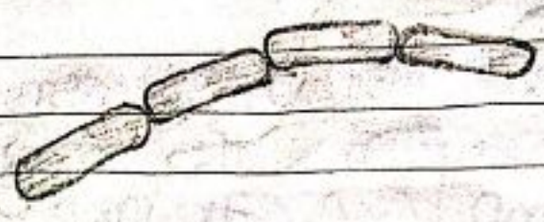
⇒ These are rod or stick-shaped cells. On the basis arrangement of an individual organism they may be;

(a) Microbacilli :- bacilli that occur singly

(b) Diplobacilli :- bacilli that occur in pairs.

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(c) Streptobacilli :- bacilli that occur in chains.



(d) palisade arrangement :- Bacilli lined side by side like Matchsticks & at angles to another.

e.g.:- Corynebacterium diphtheriae causing

↓

diphtheria.

(e) Comma-shaped :- bacilli that are curved and look like a comma.

e.g.:- Vibrio cholera causing.



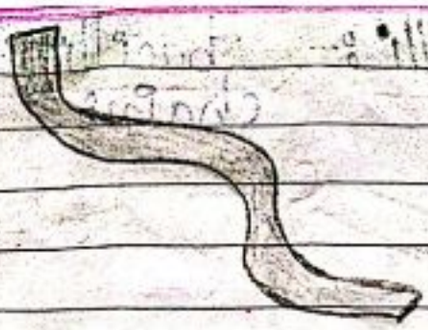
↓

cholera.

(f) Spirillum :- bacilli that are coiled like a cork-screw through 1-5 complete turns.

e.g.:- Spirillum minus causing rat bite fever.

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3. **Coccobacilli** :- These are the bacteria which are intermediate to occurs Coccus and bacillus.

e.g :- Brucella

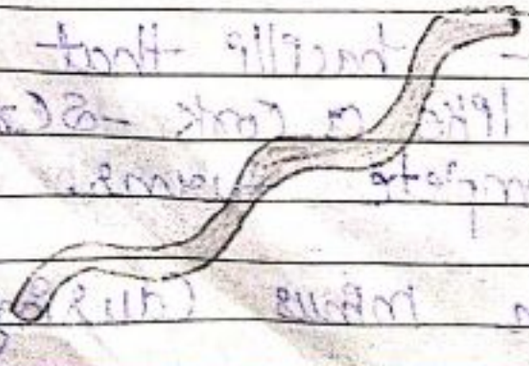
4. **Spirochetes** [Speir meaning Coil ; chaite Meaning hair].

They are relatively longer, slender, flexous, non-branded Micro organism of spiral shape having several coils.

e.g :- Treponema pallidum causing

↓

Syphilis.



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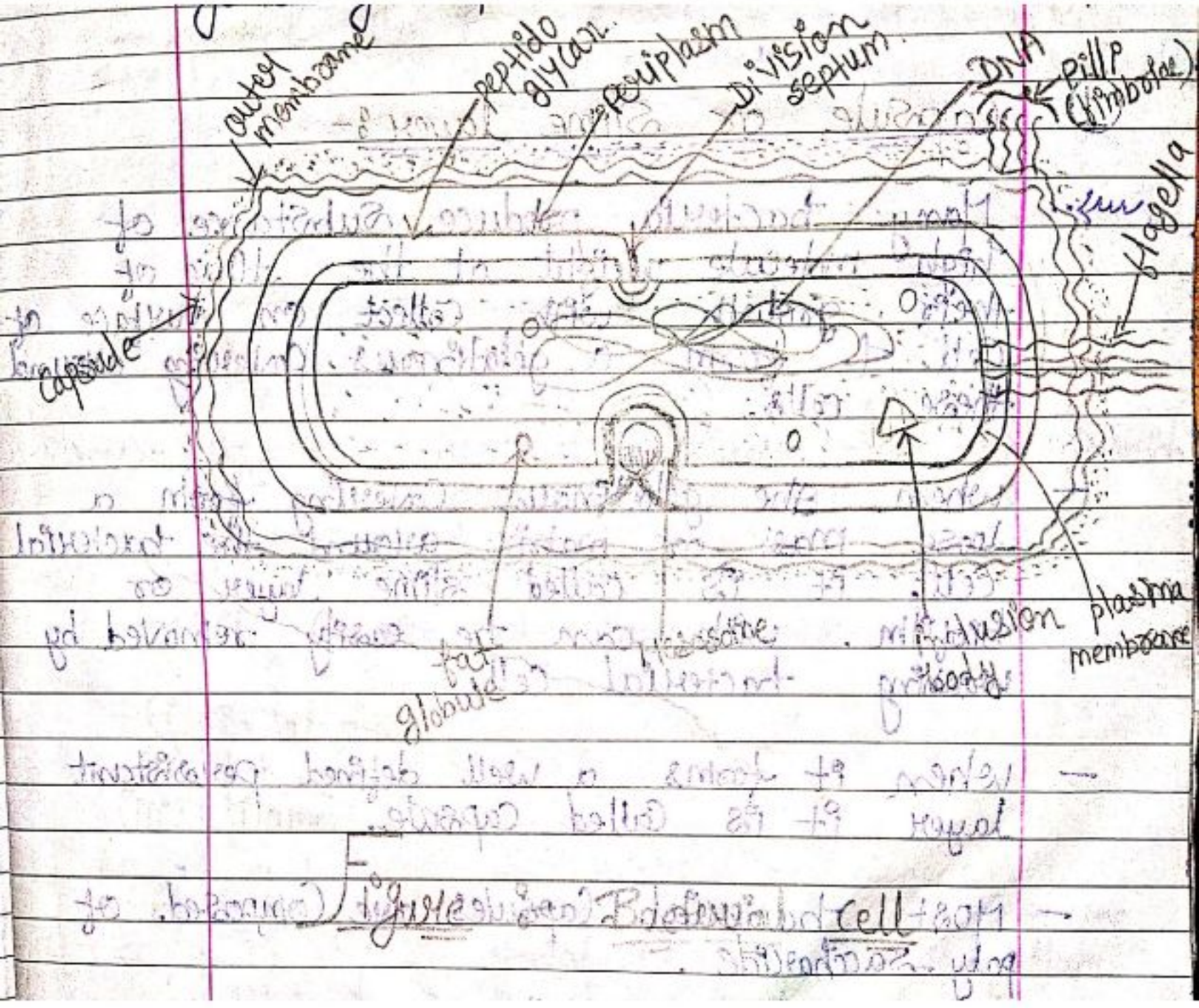


Figure: Bacterial cell

Q15. * Isolation And preservation of pure culture :-

Bacteria exist in nature in mixed forms.

To study every species independently, it is necessary to obtain their pure culture.

The two major steps of obtaining a pure culture are as follows :-

- Firstly the culture has to be diluted until the various individual micro-organism are seperated far apart on agar surface that after incubation they form visible colonies isolated from the colonies of other micro-organism. this plate is called an isolation plate.

- Secondly, an isolated colony has to aseptically picked off the isolation plate.

* Method of isolating pure culture :-

1. Streak plate Method.
2. pour plate Method.
3. Spread plate Method.
4. Serial dilution Method.

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(1) Streak plate Method :-

⇒ Most common technique of Isolation of bacterial cells on the surface of agar plate is the Streaking method.

⇒ It is a simple & rapid way of Isolation of bacterial cells on agar plate through Mechanical Means.

⇒ prepared by Streaking a small amount of Mixed culture over the surface of the solid Media in petri plate with a platinum loop.

⇒ The Sample is streaked in such a way as to provide successive dilution.

⇒ A second plate may also be streaked from the same loop without reinoculation.

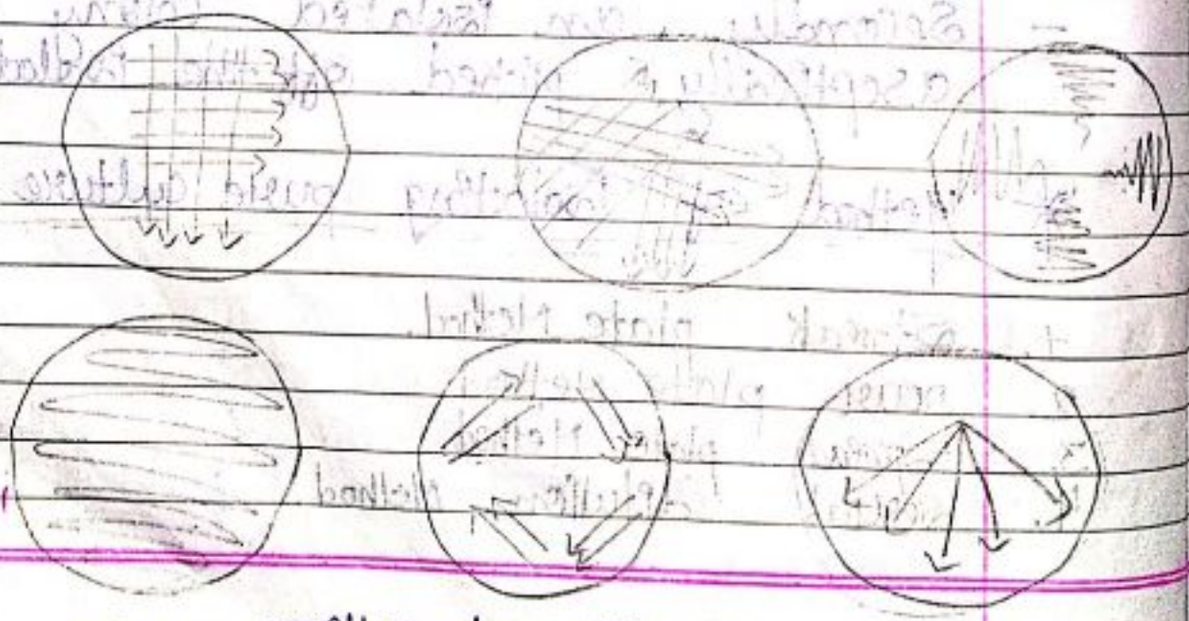


Figure:- Different patterns of Streaking.

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(2) Pour plate Method :-

- Used to Count the number of colonies making bacteria in liquid specimen.
- A fixed Amount of Inoculum is taken from a booth & it is placed in the centre of a sterile petridish with the help of sterile pipette.
- Molten Cooled agar is then poured into the petriplate containing Inoculum and Mixed well.
- After the solidification petri plate is Incubated at 37°C for 24-48 hrs.
- Bacteria will grow both on the surface and within the media.
- Colonies which grow within the medium are very small in size & may be confluent.

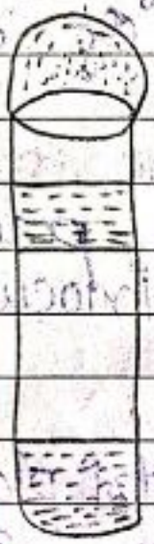
(3) Spread plate Method :-

- In this, the sample is diluted and then a little amount of it is added to the agar plate.

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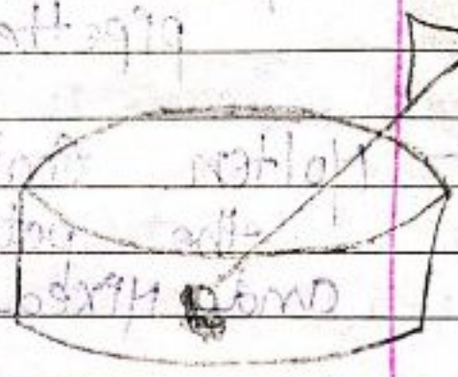
- Then the sample is spread over the agar surface evenly with the help of spreader.

- The plate are incubated and the isolated colonies are observed after 24 hrs and counted.

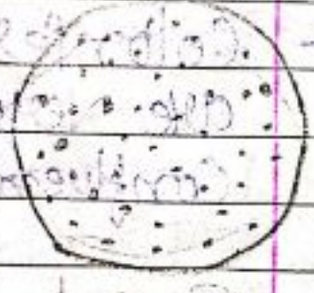


Dilute bacterial suspension

0.1ml



Nutrient agar plate



Isolated colonies

(4) Serial dilution Method :-

- Method of Stepwise dilution of a Substance.
- dilution factor is kept constant, resulting in the geometric progression of Concentration in a logarithmic fashion.
- Each dilution reduces the Concentration of bacteria by a specific amount.
- By Calculating the total dilution over the entire series, it is possible to know how many bacteria were present when the process was started.

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* Preservation of Cultures :-

⇒ To maintain an isolated pure culture for extended periods in a viable condition without genetic change is refered to as a preservation.

⇒ Various objective for preservation are :-

(1) Academic use.

(2) Fermentation Industry.

(3) Biotechnology field.

(4) Research purpose.

⇒ Various Method used for preservation are :-

(1) Periodic transfer on fresh Media :-

- Generally, Microbes are preserved on agar slants.
- The slants are incubated for 24 hours or more & stored in refrigerator.
- These culture are periodically transferred to fresh Media.

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- Time interval for transfer depend on Micro-organism and condition for growth
- Simple Method.
- Risk of Contamination which may change the genetic and Biochemical characteristic of the Cultures.

(2) Storage At low temperature :-

- Live Culture Stored in refrigerator temperature Maintained at 4°c.
- Used for short time preservation & Sub Culturing is necessary if the period exceeds four weeks.
- In another low temperature Method liquid nitrogen has provided long term preservation.
- Micro-organism are prepared as dense suspension containing protective agent [glycerol or dimethyl Sulfoxide] which prevent Cell damage due to Ice Crystal formation.
- Cell remain viable for 10 to 30 years without changing characteristics.

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(3) Storage in Sterile Soil :-

- Mainly applied for preservation of Sporulatin Micro-organism.

e.g:- Bacillus, Streptomyces.

- pure culture of Micro-organism are kept in a sterile soil medium and preserved for a number of Month under refrigeration.

(4) Preservation by overlaying culture with Mineral oil :-

- Many bacterial species can be preserved by covering their growth on the agar slant with sterile Mineral oil or liquid paraffin.

→ The oil must cover the slant complete.

- Simple Method.

- Mainly used for anaerobic Micro-organism.

- Cost effective Method of preservation of culture of bacteria and fungi

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for 15 to 20 years. but change in characteristic still occur.

(5). Lyophilization [freeze drying] :-

- A dense cell suspension placed in small Vials & frozen at -60°C to -78°C
- The Vials are immediately connected to a high vaccum line.
- Ice present in the frozen suspension evaporates under vaccum. Result in dehydration of bacteria with minimum of damage to delicate cell structure.
- Vial then sealed off under vaccum store in refrigerator.
- Remain viable for more than 30 years.
- Sub-Culturing not required. So, Contamination not occur.
- Genatically Stable.

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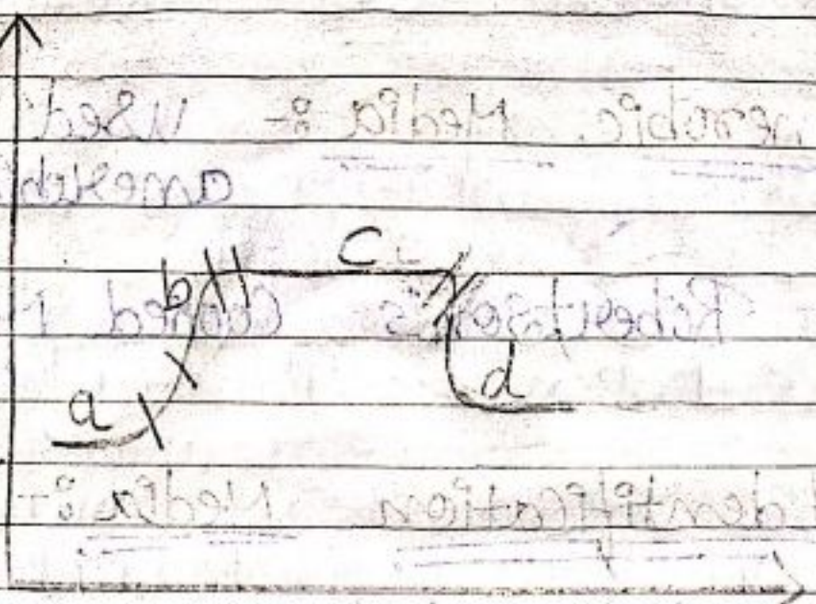
Q.16. Enumerate the condition required for the growth of Microorganism draw typical bacterial growth curve.

⇒ Condition required for the growth of Microorganism :-

1. Temperature.
2. PH.
3. Osmotic Pressure.
4. Oxygen
5. light

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* Bacterial Growth Curve



① log phase

② log phase

③ Stationary phase

④ Decline [death] phase

↳.. Growth of bacteria can be determine by Inoculating small number bacterial cell in suitable culture media & counting bacteria at regular interval.

- Bacterial growth can be Messured by ploting graph of the log of

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showing organism against time.

1. **lag phase** :- when bacteria are inoculated in fresh medium microbial population remain constant for an initial period.
   - The time between inoculation and beginning of multiplication is known as lag phase.
   - In this, phase bacterial cell adjust it self to adapt new environment.
   - duration of lag phase depend of nature of species, physicals and chemicals growth factor.

2. **log phase** :- During this phase the cell devide stability at constant rate. So, it give straight line.
   - The bacteria multiply at maximum rate & their number (res) exponentially.
   - The time required for one bacteria division called generation time.

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(3.) Stationary phase :- In this phase Constant high population Maintain by balance between cell division and cell death.

- The rate of Multiplication is reduced because depletion of nutrients, accumulation of toxic product and low partial pressure of O2.
- A Variable population Count at this Stage show no change.

(4.) Decline [death] phase :-

- During death phase no. of Viable cell decrease exponentially.
- It is Inverse of log phase.
- The Causes of bacterial death is dedation of nutrient and accumulation of toxic waste product.

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* ⇒ physical factor affecting the growth curve of Micro-organisms

Q17.

✓ (1) **Temperature** :- Temperature is most important factor that determine rate of growth.

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(a) Minimum growth temperature :-

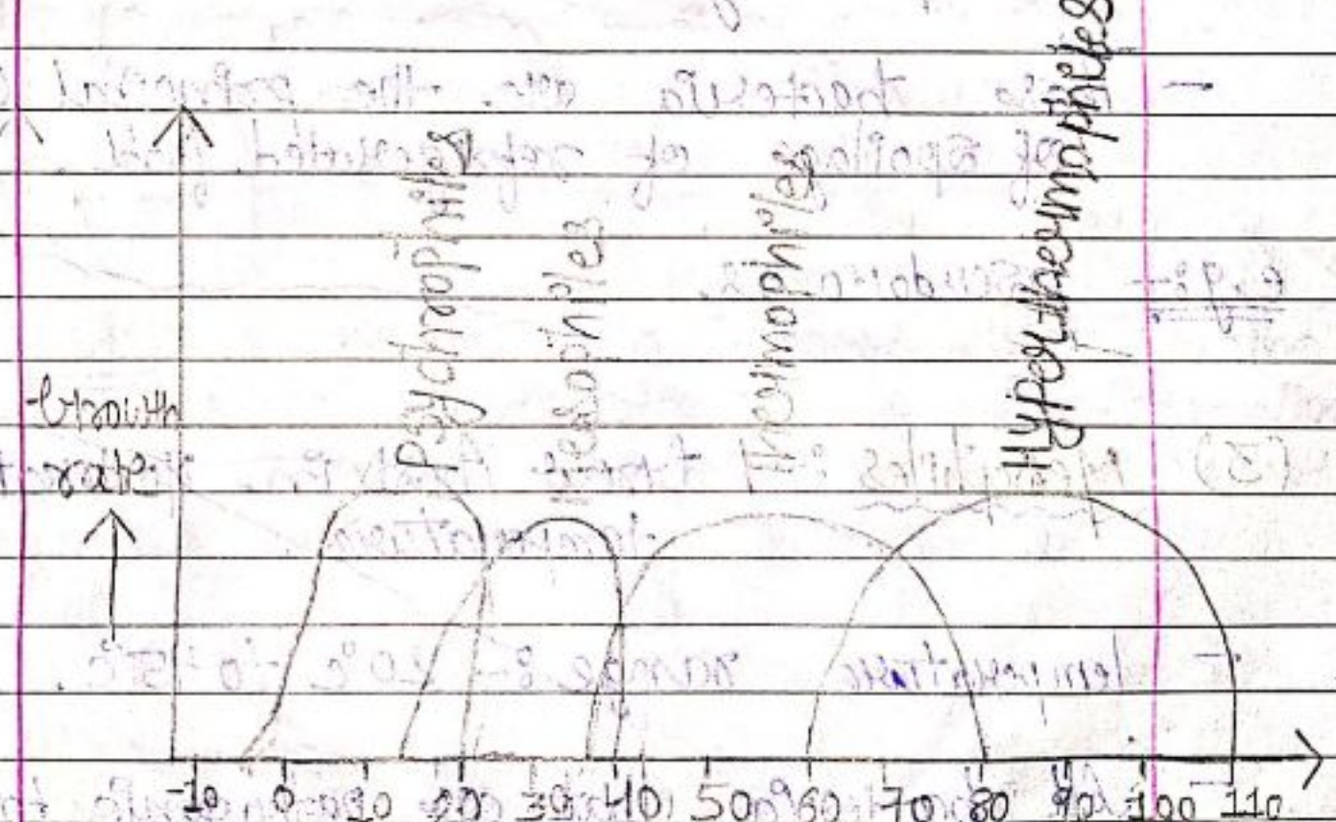
- The lowest temperature at which organism grow.

(b) Optimum growth temperature [15-20°c] :-

- The temperature that allow rapid grow in short period.

(c) Maximum growth temperature [5° - 35°c] :-

- The highest temperature at which grow occur.



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* Classification of bacterial according to growth temperature :-

(1) psychrophiles :- Able to grow at 0°C but have an optimum temperature of 15°C or lower.

- Temperature range for growth :- 20°C to 10°C

e.g :- Vibrio psychroerythrus.

(2) psychotrophs :- Can also grow at 0°C but have optimal & Maximal growth temperature above 15°C to 20°C respectively.

- These bacteria are the principal cause of spoilage of refrigerated food.

e.g :- pseudomonas.

(3) Mesophiles :- Grow best in Moderate temperature.

- Temperature range :- 20°C to 45°C.

- All bacteria that are pathogenic for human and warm blooded animals are Mesophiles.

e.g :- E. Coli.

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(4) Thermophiles :- Heat loving Micro-organism.

- Grow at 50°c or higher. Their growth minimum is usually around 45°c & often optimum between 50°c & 80°c.

eg:- Thermus aquaticus.

(5) Hyper-thermophiles :- grow in extremely hot environment.

- Temperature range 80°c to 113°c.

- The cell membrane contain high level of saturated fatty acid to retain its shape at high temperature.

e.g:- Thermotoga, etc.

(2) pH :- pH has a profound effect on the multiplication of micro-organism each microbial species has definite pH range for growth & multiplication.

- Depending upon optimum pH value of micro-organism they can be classified as follow :-

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✓. (4) Oxygen :- Oxygen is a universal component of cells & is always provided in large amount by H_2O.

* depending on oxygen requirement bacteria are classified into two types :-

(1) Aerobic bacteria :- bacteria that require oxygen for growth & can grow when inoculated in air atmosphere E.coli.

(2) Anaerobics :- They do not require oxygen for growth.

(3) Facultative aerobes (non-stringent) :-

- They are anaerobes that can grow even in the presence of oxygen. not require for growth, but if available use for energy production.

e.g:- pseudomonas species.

(4) Facultative anaerobes :- They can grow even in absence of oxygen.

(5) Microaerophilic bacteria :- Require low level of oxygen for growth but cannot tolerate the level of oxygen present in an air atmosphere.

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Nutritional Types of bacteria :-

- Autotrophic bacteria.
  - photoautotrophic bacteria
  - chemoautotrophs.
- Heterotrophic bacteria.
  - photoheterotrophs.
  - chemoheterotrophs.

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① Autotrophic bacteria :-

ws.. bacteria with are able to synthesize their own organic food from inorganic substances supplied from environment.

- distinguished further into two types :-

(a) photoautotrophic Bacteria :- Utilize Solar energy to Synthesize their organic Metabolite.

- possess photosynthetic pigment in Membrane bound lamellae.

(b) chemoautotrophs :- Manufacture organic compound from inorganic raw Materials and utilize energy liberated from the oxidation of these inorganic substances.

② Heterotrophic bacteria :-

ws.. The bacteria with are unable to Manufacture their own organic food & hence are dependent on external source are called heterotrophs.

(a) photoheterotrophs :- Organisms that use light for energy. but cannot use Co_2 as their carbon sources they use organic compound [carbohydrate,

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fatty acid] from the environment to satisfy their carbon requirement.

(b) Chemoheterotrophs :- They cannot synthesized their own food, they obtain it directly from external environment using organic compound.

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★ [3] Filtration Method [Mechanical Method] :-

Q.18.

⇒ In this Method, Microbes are just separated out from the product by means of filtration.

- filtration removes the Microbes but doesn't.

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kill them.

∴ It is a non-thermal Method of sterilization used widely in pharmaceutical industry for thermolabile substances.

→ filtration efficiency is depend upon....

- pore size of media.
- Thickness of wall.
- filtration rate.
- Nature of the liquid to be filter.

⇒ On the bases of type of filter media, there are Main four types :-

(a) Asbestos filter [Seitz filter].

(b) Sintered glass filter [Morton filter].

(c) filter Candles [ceramic / berkefield filter].

(d) Membrane filter [Millipore / ultra filter].

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[a] Asbestos filter :- [Seitz filter]

- In this Method, disposable and single use disc Made up of Asbestos [Magnesium trisilicate] are used.
- It is supported on the perforated disc within a Metal funnel.
- Then, it is fitted on to a sterile flask through a silicone rubber tubing.

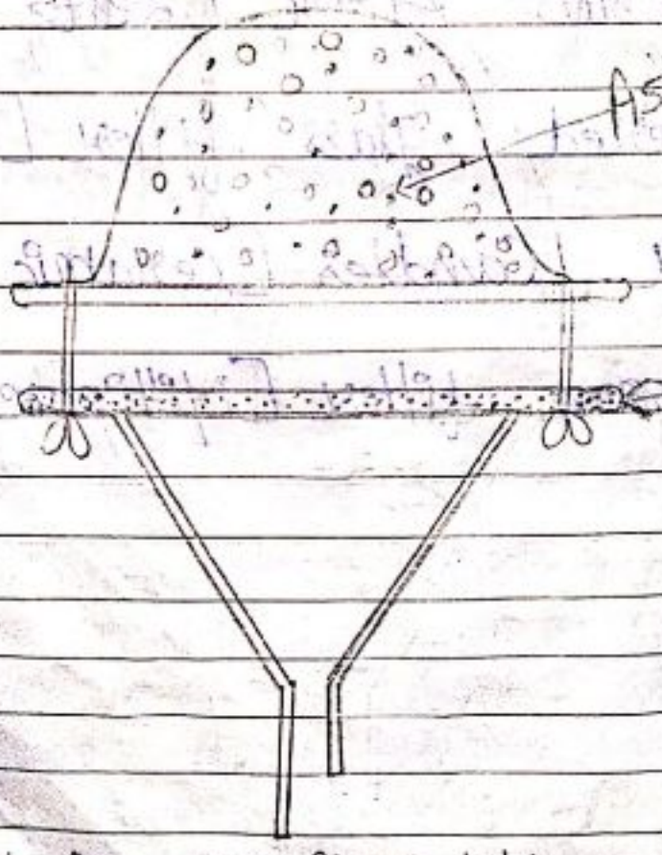


Figure :- Seitz filter.

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- fluid to be sterilize is put into the funnel and flask. is connected to the exhaust pump.
- after completion of filteration, filtrate is collected and filter is discarded.
- pore size of asbestos filter :- 0.01 to 5μ.

[b]. Sintered glass filter :-

- It is also known as → Morton filter or fritted glass filter.

⇒ preparation of Sintered disc :-

- Borosilicate glass is finely powdered in ball mill and packed into disc mould.
- Now, heat it until suitable adhesion take place between the granules.
- Now, fuse the prepared sintered disc into funnel of a suitable size & shape.

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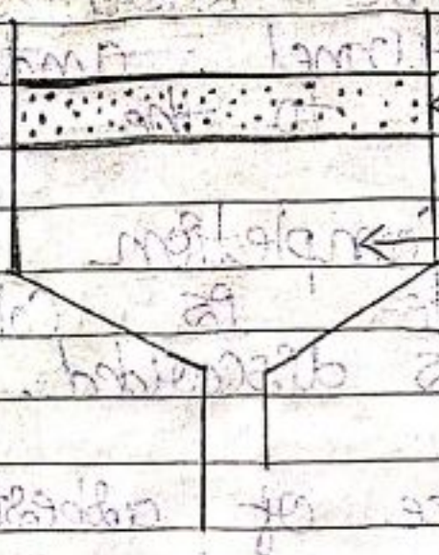


Figure :- Sintered glass filter.

- Sintered glass filter have low adsorptive properties and can be clean easily.

disadvantages :- - Brittle and expensive.
- Small area of filtration.

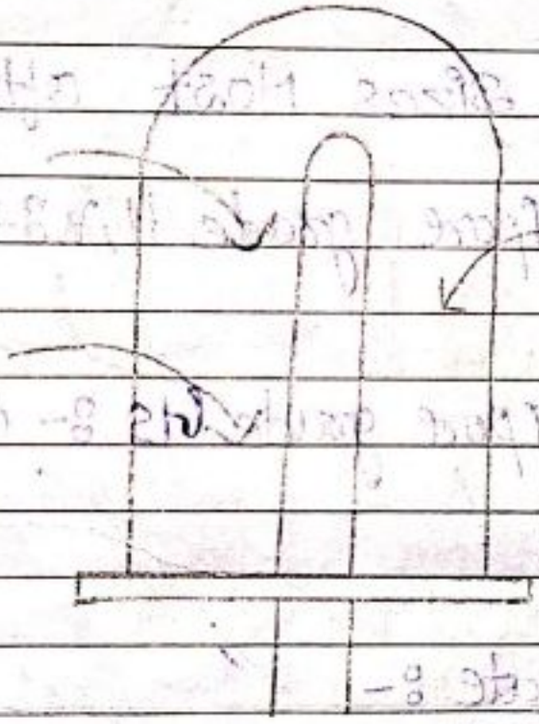
[c] Filter Candles :- [Ceramic / Berkefeld filter].

- These are Manufactured in different grades of prosity.
- filter Candle are Made up of either porous porcelain or kieselguhr.
- It looks like Cylindrical Candles with comparitive thick walls.

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* operation :-

- The filter is fixed to the filter assembly and placed on a Mantle.
- The liquid to be filtered is poured into the mantle where vaccum forces it through the filter.
- after filtration, filter Candle is removed from the assembly.
- filterate is transfered into Sterile Container.



Advantages :- ⇒ It is cheap.

⇒ available in different sizes.

Disadvantages :- ⇒ easily clogged and blocked.

⇒ high pressure is required.

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[d]. Membrane filter :- [Millipore Ultrafilter]

- They are Made up of various types of cellulose and cellulose esters.
- Thickness of filter is 150 um.
- It Contains Millions of Microscopic pores.
- pore Size range is 0.01 to 10 um in diameter.

⇒ The pore sizes Most often used are:

- (i) Millipore grade HA :- 0.45 um ± 0.2 um.
- (ii) Millipore grade GS :- 0.22 um ± 0.2 um.

⇒ filtration rate :-

(i) The HA grade :- 65 ml / min / cm².

(ii) The GS grade :- 22 ml / Min / cm².

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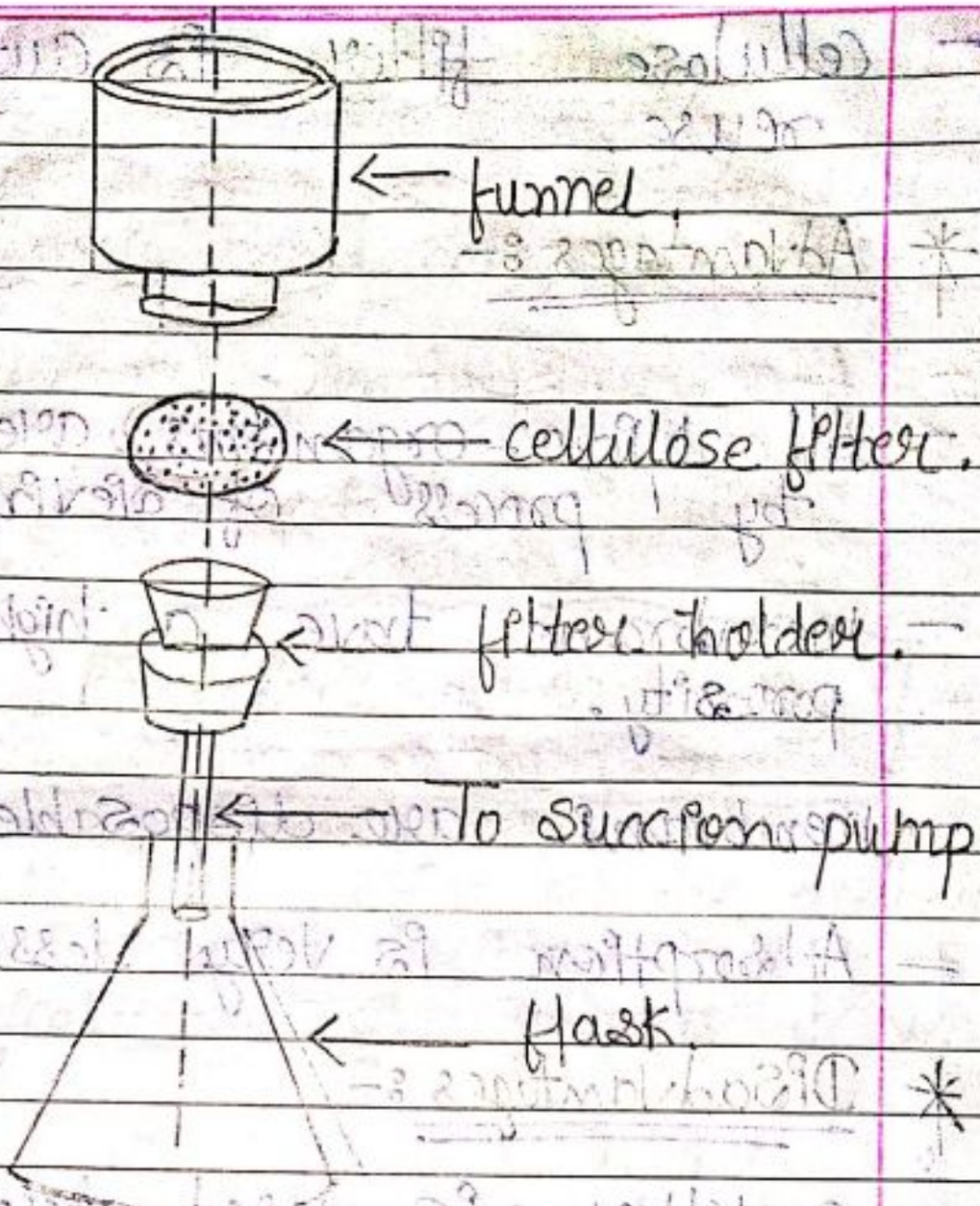


Figure :- Membrane filter.

- In this Method, membrane filters are supported on a rigid base of perforated Metal or plastic.
- Solution or liquid to be sterilize are powred to funnel and filter through cellulose membrane.
- filtrate is collected in sterile flask.

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- Cellulose filter is autoclaved for reuse.

* Advantages :-

- All Micro-organisms are separated by process of sieving.
- membrane have a high and uniform porosity.
- membrans are disposable.
- Adsorption is very less.

* Disadvantages :-

- prefilter is used before filtration to avoid clogging and breaking.

* Application :-

- Routinely used in water purification.
- Analysis, sterilization & sterility testing & for the preparation for parenteral use.
- used for the identification & enumeration of Micro-organism from water sample & other material.

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Q-19.

(5) Hot air oven :-

Principle :-
- Most widely used Method.
- Same as dry heat.
- Oxidation of cell Content.
- protein denaturation.
- Toxicity caused due to high electrolyte Content.

Instrument :- Hot air oven is Mainly Consist of following parts :-

(i) Metal chamber :- The oven Consist of an aluminium or Stainless steel chamber with the door Covers an asbestos gasket to provide tight Seal.

(ii) Heating Coils :- It Can be placed in the side walls or under the floor for best distribution of steam.

(iii) perforated plates :- It holds the Material or article to be dry/ Sterilize.
- due to perforation in it helps in efficient circulation of hot air.

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(iv) Fan :- Fan or turbo blower fitted at back wall helps to circulate the air all over the oven.

(v) Digital analyzer :- It helps to analyze the temperature kept inside the oven with the help of temperature probe or thermometer.

Figure :-

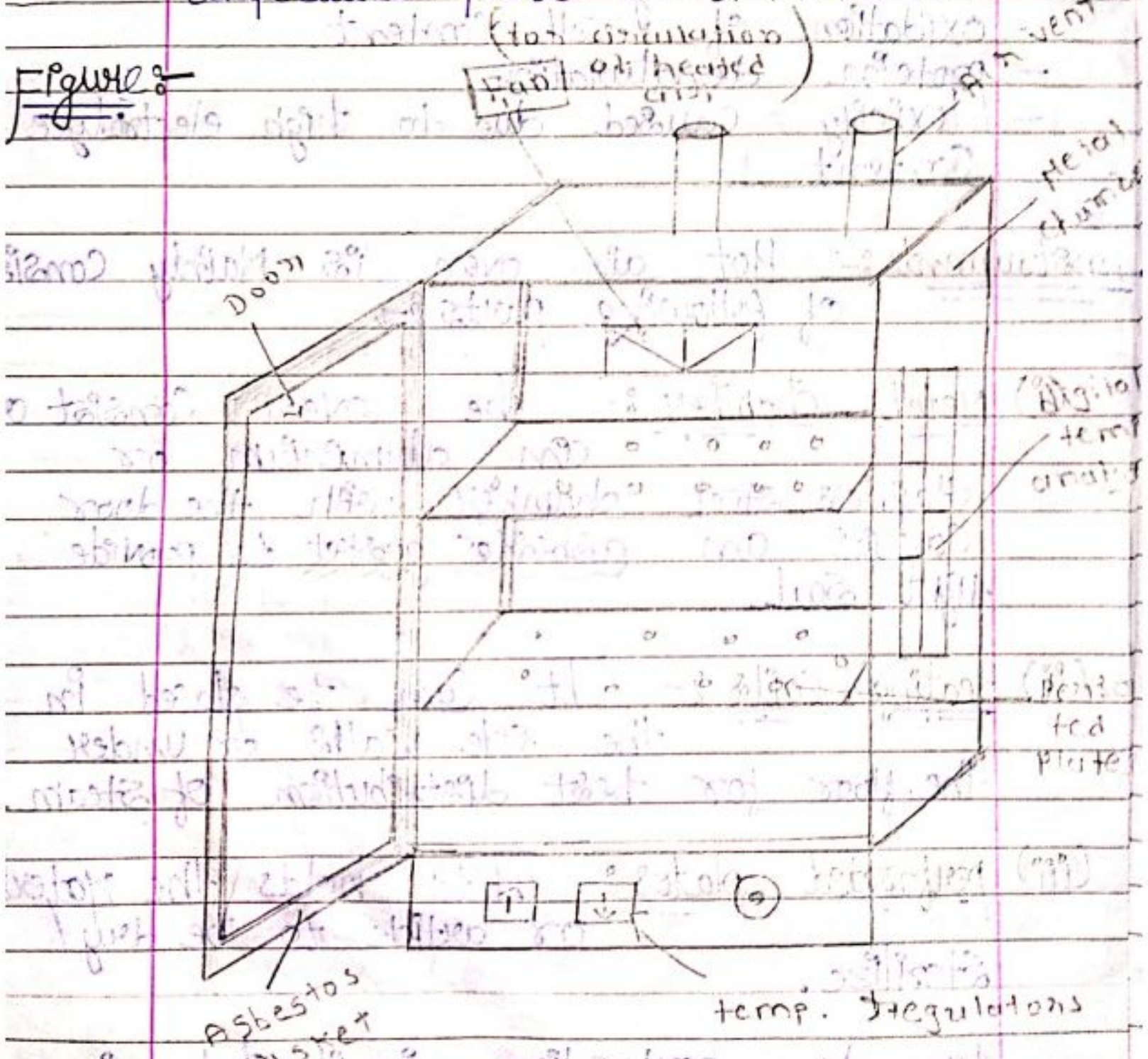


Figure :- Hot air oven

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* Sterilization monitors :-

(1) Physical :- use of thermocouple & temperature are recorded.

(2) Chemical :-

— Browne's tube No. 3 (green spot) is used for this purpose.

Indication :- when an appropriate temperature is attained colour change from red to green.

(3) Biological :-

— B. subtilis var. niger
— B. stearothermophiles

} loaded on strip & placed inside hot air oven.

after completion of cycle,

strips are removed.

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↓

Inoculated into thioglycollate broth.

↓

Incubated at 37°C for 3-5 days.

↓

If there is no growth,

↓

it indicated proper sterilization.

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Operation :-

- Articles to be sterilized have wrapped with suitable materials such as paper and place into hot air oven. or enclosed in Containers.
- for, proper sterilization oven should be properly loaded such that,.....
- each article can receive Maximum possible heat.
- Vent should be closed & the door can then be shut & fan is switched on.
- Temperature variation within the chamber should not exceed ≥5°C of recorded temperature.
- The heat is transfered to the article by radiation, Conduction and Convection.
- The relation ship between the temperature and relative time required for sterilization is given in table :-

Boiling Point	Temperature	EXPosure
Oily Solution.	150°C	1 hr
glass ware.	160°C	1 hr
B.P.C Glassware.	180°C	11 Minutes
U.S.P glassware.	180°C.	2 hr.

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- The oven must not be opened until the temperature falls below 60°C to prevent breakage of glassware.

* Advantages :-

- Suitable for the Materials that are damag by Moisture. [oils and powders].
- It is useful for the assemble equipment since, Sufficient time for penetration.
- Very easy to handle and economic.
- less damaging to glass & Metal equipment than to Moist heat.
- does not leave any chemical residue.

* disadvantages :-

- dry air is poor conductor of heat and penetration powder is very low Compare to Moist air.
- High temperature & long exposure. So, medicament, rubber & plastic are not Stabled.
- Hence, It takes longer time Compare to autoclave.

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- Can not be used for articles that contains alcohol, or volatile liquid which is evaporated at high temperature.

⇒ Not suitable for surgical dressing as natural Moisture vaporises it.

- Takes longer time as compared to autoclave.

Application:- Hot air oven is used to sterilize.

- glassiwares [flasks, beakers, pipette, petridish.
- equipment [scissors, scalpels, Motor pestle, Surgical instrument];
- powder [Talc, kaolin, starch, lactose, ZnO].

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Q20

[A]. **Autoclave**

Principle: As the pressure inside closed vessel increases, the temperature also increases.

↓

reaches to sterilizing point.

↓

At a pressure of 15 lbs inside autoclave.

↓

temperature reaches to 121°C

↓

for 15 Minutes.

↓

And this way protein gets denatured and Coagulant.

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2. Heat in the form of Saturated steam under pressure.

↓

Is Most practical & dependable agent for sterilization.

↓

Stem under pressure provides Temperature above those Obtainable by boiling.

* Mechanism of action:-

- Denaturation & Coagulation of bacterial protein.
- Bacterial protein —High Moisture Content→ -SH group.

↓

form small peptide chain.

↓

forms a new Complex with different from original protein.

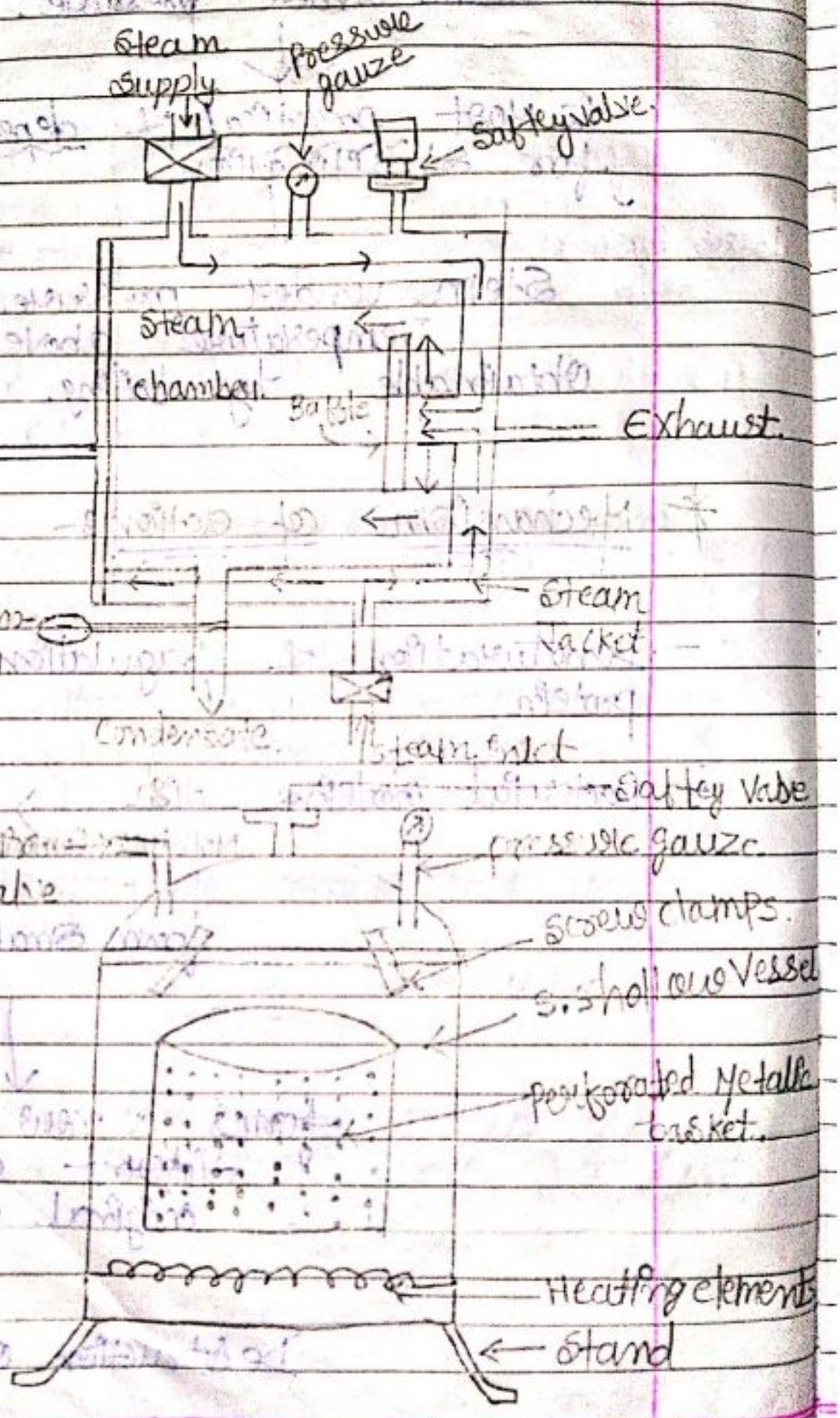
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Destruction of bacteria.

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* Equipment Design :-

[Figure :- Horizontal Autoclave]



[Figure :- Vertical autoclave.]

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* Construction :-

- It Consists of steel cylindrical hollow vessel which contains perforated Metallic basket in it.
- Body of cylindrical vessel is closed with lid and fitted with screw clamps.
- There are three controls present on the lid :-

① Air vent.
② Safety valve.
③ pressure gauze.

- The electric heating element is fitted at bottom of autoclave.

* Operation :-

- The bucket full of water added to immerse the heating element completely.

↓

The Material or articles; to be sterilized are loaded into perforated basket.

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The lid is tightly closed and source of heat is turned on.

↓

allowed to escape air mixture till the cylinder becomes air free.

↓

air vent is kept open and safety valve is set at the required pressure.

↓

Vent is closed when steam is covering freely from vent for 5 min.

↓

The steam pressure rise in vessel & the temperature (rise) rise.

↓

reach to sterilizing point.

↓

from this point holding time is counted and when the holding time is over, the heating is stopped .....

↓

and autoclave is allowed to cool down

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till the pressure inside Vessel reaches to atmospheric pressure. [indicated by pressure gauze].

↓

finally, the lid is opened and the Sterilized articles are removed.

⇒ The whole operation requires four condition :-

ⓐ adequate Contact

ⓑ proper time of exposure.

ⓒ Sufficient high temperature.

ⓓ Sufficient Moisture.

⇒ The time of operation to achieve Sterility depends on :-

① Nature of Material being sterilized.

② Type of Container.

③ The volume.

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Temperature (°C)	pressure (PSI) Pound/Square Inch	Holding time (Min).
115 (B.p)	10	30
121 (VSP)	15	15
126°C	20	10
133°C	30.	3

Table :- Sterilizing Condition for an autoclave.

* Advantages :-

- Penetration power of steam is very high. So, Microbes are killed more efficiently in lower time.
- large quantity of materials can be sterilized.
- Sealed containers and ampoules are readily sterilized.

* disadvantages :-

- Not suitable for oils, ointment, powder, and oily injection.
- plastic items are not sterilized because can't withstand high temperature.

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- requires Continuous monitoring of time temperature and pressure.
- difficult to produce dry packs. (Due to Moist).
- It is unstable for Sterilization of powder & oil.

* Application :-

⇒ This Method is use to Sterilize,

- Screw clavies.
- Rubber Closures.
- Suspen it.
- Injection Solution.
- Surgical dressing.
- certain ~~matt~~ Copy with equipment, etc.

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