2 Marks questions

1. Define Microbiology. Explain different applied branches of microbiology.
2. Different b/w prokaryotic cells and eukaryotic cells.
3. Draw the structure of prokaryotic cells.
4. What is the contribution of Louis pasteur in the field of microbiology.
5. Write the properties of Agar.
6. Draw a structure of bacterial cells.
7. Different between flagella and pilli.
8. Write the function of pilli.
9. Write the function of capsules.
10. ~~With~~ Write function of cell wall in bacteria.
11. Differentiate between total count and viable count.
12. Write advantages and disadvantages of plate count method.
13. Write short note on growth curve.
14. Write two limitation of electron microscopy.
15. Write the principle of simple staining.
16. Define staining?
17. Define sterilization.
18. Write advantages and disadvantages of moist heat sterilization.
19. Write short note on —
    i) Ethylene oxide. — (2)
    ii) Hot air oven — (2)
    iii) Membrane filter — (2)
20. Write the importance of fungi.
21. Differentiate following :—
    a) Moulds and yeast — (2)
    b) fungi and bacteria — (2)
    c) bacteria and virus — (2)
22. Difference b/w DNA Viruses and RNA viruses.
23. Draw a structure of T-even bacteriophage.
24. Define disinfectants.?
25. Write ideal properties of a disinfectants.
26. Write action & mechanism of following disinfectants :-
    a) phenol — (2) b) Alcohal — (2) c) aldehyde - (2)

27. What is MIC ?
28. What media are used for steility testing?
29. What do you understand by IMViC ?
30. What is pasteurization?
31. What is cold sterilization
32. What is Aseptic technique ?
33. What is heterotrophic bacteria.
34. What is Acid-fast staining
35. What is the contribution of Alexandar flemming in microbiology
36. Enlist the vegetative modes of reproduction in fungi.
37. Enlist different types of phenol coefficient tests.
38. What is microbial spoilage
39. Difference b/w Bactericidal and Bacteriostatic.
40. What are sterility indicators

IMPORTANT QUESTIONS
OF
PHARMACEUTICAL MICROBIOLOGY

ANSWERS/SOLVED

① Microbiology ⇒ It is the branch of science, which deal with the study of small living organism, which is less than 1 H(micron) approx.

eg Viruses, Bacteria, fungi -- etc..

- Branches →

Virology → study of virus.

Bacteriology → study of Bacteria

Protistology → Study of Protist.

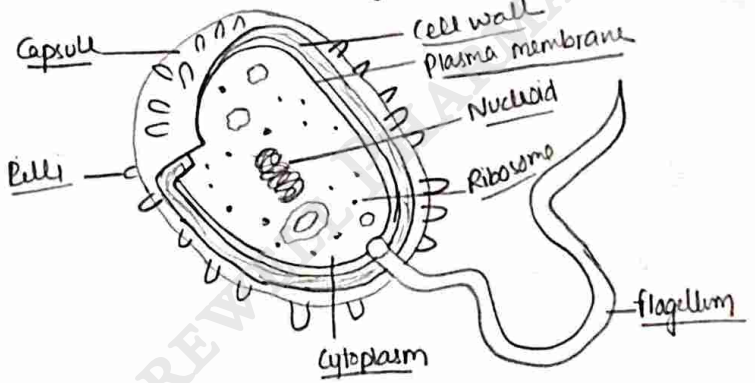
Mycology → study of fungi.

②

Eukaryotic Cells	Prokaryotic Cells
- Those cells in which cell membrane, Nucleus their organelles are fully developed.	- Those cells in which cell membrane developed, but nucleus membrane does not developed, also some organelles not developed.
eg fungi, Algae, Nematoda	eg Bacteria, Protozoa

③ Structure of Prokaryotic cells

⇒



④ Louis pastur → He is best known for inventing the process pasteurization.

- Pasteurization kills microbes and prevent spoilage in beer, milk and other goods.

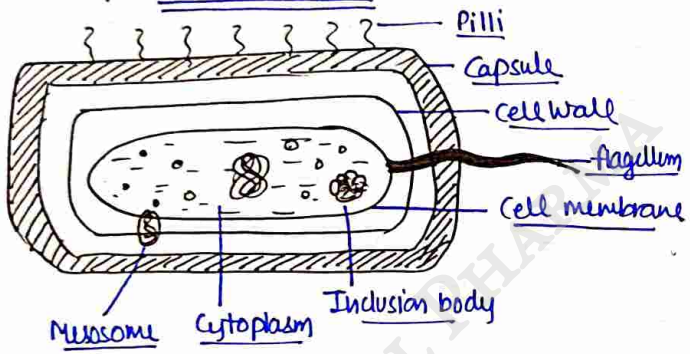
- He is commonly dubbed the "father of microbiology".

(5) properties of Agar..-

⇒ • Agar is usual solidifying agent for media.
• properties → melting point 100°
Remains liquid until cooled to 40°.
Cannot be metabolized by most bacteria

(6) Structure of bacterial cells

⇒



[TRY TO USE PENCIL FOR DIAGRAM IN EXAM]

(7)

Flagella	Pilli
• It is 15-20 nm long thick hair like structure	• It is small thin 8-10 hair like structure
• It work like as antenna, which give signal to bacteria	• It helps in attachment of bacteria with other bacteria

(8) function of pilli-- (2)

⇒ • It is small thin 8-10 hair like structure
• It helps in attachment of bacteria with other bacteria
• Also helpful in transfer in genetic material

(9) Write the function of Capsules..

⇒ • It gives protection to the bacteria, also give shape and size to bacteria also prevent from phagocytosis.
• It causes disease in human body
• It helps in attachment and repulsion with other bacteria

(10) Cell wall → It is also thick structure made up with peptidoglycan layers.
• It provide protection, shape and identification to the cell bacteria
• It can also helps in identification of bacteria (gram (+) or gram (-)).

(11)

⇒ . Total count ⇒ In this count, we have to count all bacteria (either it live or deid).

- method used for this :-
  - counting chamber method.
  - Electron counter method.

• Viable count ⇒ In this count, we have to count only those bacteria which are alive, also grow regularly

- method used for this :-
  - Plate count method
  - Membrane filter count method

(12) Advantage and disadvantages of Plate count method :-

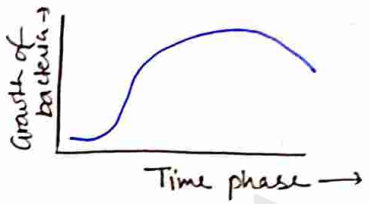
⇒ Advantages → • Measurable viable cells
• Simple, useful and sensitive
• Gives count for viable cells

Disadvantages → • Take 24 hours or more for visible colonies to appears
• only count b/w 25 and 250 colonies are accurate.
• Must perform serial dilutions to get appropiate no/plate

(13) Write short note on growth curve. (3)

⇒ When a graph plot between the growth of bacteria and time phase is called growth curve

- The resulting curve has four distinct phases :-
  - Lag phase
  - Log phase
  - Stationary phase
  - Decline phase



(14) Electron Microscopy →

~~The main disadvantages are cost, size, maintenance, researcher training and image artifacts resulting from specimen preparation, typically about 1 nm~~

• They are extremely expensive and also maintenance cost is high.
• It is not possible to observe moving specimen (they are dead).
• Dead specimens only → The energy of electron beam is very high. The sample is therefore exposed to high radiation, and therefore not able to live.

(15) Principle of Simple Staining :—

⇒ firstly developed the (-)ve on the surface of bacteria, then add (+)ve charge dye on it Dye attached on bacteria easily.

- When dye attached on the surface of bacteria, Bacteria will visible easily in also light background.

(16) Staining :— (Identification of bacteria)

- It is a ~~staining~~ technique in which we identified the bacteria, by using different technique

eg. Simple staining, Gram staining and Acid-fast staining

(17) Sterilization :— It is a process in which we remove or kills the all types of microbes (microorganism) such as bacteria, viruses, fungi etc— from the surface of all living or non-living things.

- methods used :— physical, chemical gasses, radiation and mechanical method.

(18) Advantages & Disadvantages of moist heat sterilization (4)

⇒ Advantages :—

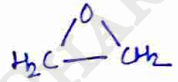
- It is very effective
- It is quicker in heating up the exposed articles.

Disadvantages :—

- It is not suitable for anhydrous ~~moist~~ materials such as powders, oils, fats etc..
- It is not suitable for heat labile substance, which cannot with stand heating at 115°c & above

(19) Write a short note on :—

⇒ • Ethylene oxide →



- It has high penetrating power and it attack on the DNA and RNA of bacteria directly and react with it.

• Hot air oven → It is a device, which is used for the sterilization.

- In Hot air oven, maintain the high temp. So the protein of bacteria are denatured or oxidised and the bacteria kills.

• Membrane filter → It is a method which is used for sterilization, also for sterility testing.
• It comes under mechanical method of sterilization.
• Membrane filters are thin porous sheet structures composed of cellulose esters or similar polymeric materials.
• It is used for filtration, in which bacteria retained on it. (products → anoil, ointments etc.)

(20) Write the importance of fungi
⇒ • fungi are responsible for breaking down organic matter and releasing carbon, oxygen, nitrogen and phosphorous into the soil and the atmosphere
• fungi are essential to many household and industrial process such as making of bread, wine, beer and certain cheese.

(21) Differentiate :—
⇒ a) Moulds and yeast → Both belong to the kingdom fungi, But yeast is single celled organism which reproduce asexually (binary fission & budding), whereas Mould are multicellular reproduce sexually or asexually

b) fungi and bacteria →

Bacteria	fungi
Prokaryotes	Eukaryotes
Unicellular organism	Both but most are multicellular
Size 0.5 - 5.0 μm	Size 2 - 10 μm
cell wall is made up of peptidoglycan	cell wall is made up of chitin.
Transmission occurs through contact, body fluids, foods, water, insect or air	transmission occurs through spores

c) Bacteria and virus →

Bacteria	Virus
Bacteria do not need a host organism for reproduction.	Virus replicate only inside the host.
Considered as living organism.	Not considered as living organism
Unicellular	Dont have cells

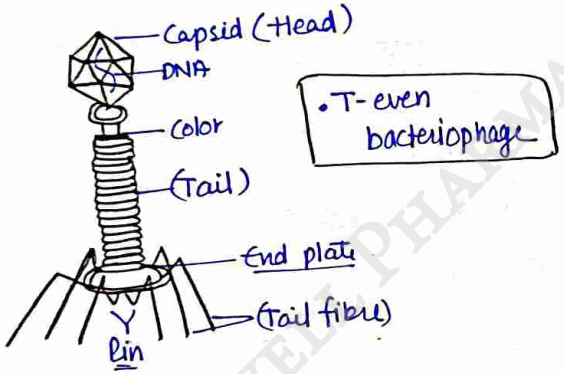
(5)

(22)

DNA Viruses	RNA Viruses
• Refer to viruses whose genetic information is stored in the form of DNA	• Refer to virus whose genetic information is stored in the form of RNA.
• Most are double-stranded	• Most are single stranded.

(23) T- even bacteriophage (structure).

⇒



(24) Disinfectant:- These are antimicrobial agents, that are applied to non-living object to destroy microorganism and the process is known as disinfection.

eg: Hydrochloric acid, phenol, Ethanol, methanol etc.

(25) Ideal properties of disinfectants :-

An ideal disinfectant should have the following properties:-

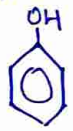
- Broad spectrum
- Non toxic
- Easy to use
- Odourless
- stable on storage

(26) Write action and mechanism of:-

a) Phenol ⇒ They destroy the microorganism by the process of disruption of cells, precipitation of cell protein.

- some important phenol and derivatives used as a disinfectant:-

phenol, o-cresol, m-cresol etc.



(phenol)

b) Alcohol ⇒ Alcohols are protein denaturants. they may damage lipid complexes in the cell membrane, they are also dehydration agents.

eg ⇒ Methanol (CH_3OH), Ethanol (CH_3CH_2OH) etc.

c) Aldehydes ⇒ formaldehyde (HCHO) is the main aldehyde used for disinfection.

- It inhibits the activity of the enzyme in the cell, denature the cell proteins.

(27) MIC ⇒ (Minimum Inhibitory concentration)

- It is the lowest concentration of antimicrobial compound found to inhibit the growth of a particular test microbial microorganism.
- It may applied to acess new disinfectants, antiseptics, preservatives and antibiotics

(28) Media used for sterility testing

⇒ Culture media used for this :-

i) fluid thioglyconate → for anaerobic bacteria and also for some aerobic bacteria

ii) Soyabean casein digest → for fungi and aerobic bacteria.

(29) IMViC → This is the biochemical series of test, which is performed for differentiate bacteria such as enterobacteria

Test performed :-
- I = Indole test
- M = Methyl Red (MR) test
- Vi = Voges - Proskauer (VP) test.
- C = Citrate utilization test.

(30) What is Pasteurization

⇒ It is a heat-treatment process that destroys the pathogenic microorganism in certain foods and beverages

eg. pasteurization of milk to make it safe for consumption and improve its keeping quality.

(31) What is Cold sterilization.

⇒ • It is a process of disinfecting instruments/ equipments by using a liquid chemical germicide is called cold sterilization.
- It is used for heat sensitive instrument.
- Heat not used in this method.

(32) What is Aseptic technique

⇒ Aseptic technique are used to prevent the acess of microbial and particulate contamination into ophthalmic and parental products
- An aseptic area is a room within a clean area designed, constructed, service and used with the intension of preventing microbial contamination of the products

(33) What is heterotrophic bacteria?

⇒ Heterotrophs are a group of microorganism that use organic carbon as food, and are found in every type of water.

eg Yeast, moulds and bacteria—

(34) What is Acid-fast staining.

⇒ . Acid fast staining are used to differentiate acid fast organism such mycobacteria.

. Acid fast bacteria have a high content of mycolic acids in their cell walls

• Acid fast bacteria will be red, while nonacid fast bacteria will stain blue/green with the counterstain with the kinyoun stain.

(35) Alexander fleming :-

- He was a scottish physical and microbiologist, best know for discovering the enzyme lysozome and the world's first broadly effective antibiotic named Penicillin.

- He receive Noble prize for this.

(36) Enlist the vegetative modes of reproduction in fungi.

⇒ Vegetative spore modes of reproduction are asexual reproduction of fungi, in which three reproduction occurs--

i) Blastospores (reproduction by budding)

ii) Arthrospores (reproduction by segmentation & condensation).

iii) Chlamydospores (thick walled resting spores developed by rounding up and thickening of hyphal segments.

(37) Enlist different types of phenol coefficient tests.

⇒ This test is used for ~~identification of~~ evaluation of disinfectants:—

. It basically involve two tests:—

i) Rideal-walker test (RW test)

ii) Chick-Martin test.

(38) Microbial Spoilage :- It is spoilage of any pharmaceutical products or drugs due to contamination of microorganism and their products, which further not intended for use eg. microbes such as Bacteria, fungi, virus etc—

(39) Difference b/w Bacteriocidal and Bacteriostatic

⇒

Bacteriostatic	Bactericidal
• Those antimicrobial agent which inhibit the growth of microbes (bacteria).	• Those antimicrobial agent which are capable of killing microbes (bacteria).
eg. tetracyclins, chloramphenicol etc	eg Betalactam antibiotics etc

(40) What are sterily Indicators.

⇒ These are those indicators that are used for check the quality of sterilization & monitoring of the sterilization process

- It may be also required to check whether microbial growth occur or not in terms of sterilization quality.

eg:- Chemical indicators → Browne's tubes, Witness tube etc.

IMPORTANT QUESTIONS

OF

PHARMACEUTICAL MICROBIOLOGY

UNIT-Ist

1. Write the history and scope of microbiology.
2. Differentiate b/w Prokaryotes and Eukaryotes.
3. Write in details about isolation of pure cultures.
4. Describe the structure of bacteria in details with the help of diagram.
5. Write a note on principle and application of Electron microscopy OR Phase contranst microscopy.
6. Write a note on Growth Curve
7. ~~01~~ factor affecting bacterial growth and measurement of bacterial growth [total & Viable count].
8. Describe in details about culture media

OR

Write a short note on anaerobic culture midia.

UNIT-IInd

1. Write in details about staining - (10)
   - Simple staining
   - Gram staining [Most Impotant]
   - Acid fast staining (Ziehl-Neelson staining).
2. What is steilization? What are various methods of steilization. Describe in details - (10)

[OR]

Describe the process the steilization by autoclaving [Details].

[OR]

Different between Dry and moist heat steilization.

3. Different b/w Gram positive and Gram negative staining.

UNIT-3

1. Write in details about Virus or Fungi
   - morphology, classification, reproduction/replication, Cultivation.
2. Briefly explain the method for carrying out the Sterility test on pharmaceutical products.
3. What are disinfectants ? Add a note on evaluation of disinfectants...
   - Phenol coefficient (Chick martin, RW) - Imp.

UNIT-4

1. Discuss the design and layout of an aseptic area
2. Discuss the principle and methods of microbiological assay
   - Vitamins (Vit B_{12} or Streptomycin).
   - Antibiotics (Penicillin).
3. Write various air flow pattern in laminar air flow

UNIT-5

1. Describe in details about animal cell culture. (preparation, maintainance, procedure, applications).
2. What are preservatives (antimicrobials) ? Discuss their role in pharmaceutical products.
3. What is microbial spoilage and its types

OR

Elaborate various factor affecting microbial spoilage

4. Write a short note on preservative efficacy test.

Pharmaceutical Microbiology

Unit = I st

Q-1 = Write the history & scope of microbiology?

Ans = It is the branch of science in which we have to study about the small living organisms which is less than 1 micron which we can't see with naked eye, is called microbiology.

• History of Microbiology:

1 = Father of Biology is Aristotle.
→ Biology is divided into two types:
a) = Living, (b) = Non-living.
→ Many living we cannot see.

2 = Father of Dieseases is Roger Becon. in 13th century.
→ Anything which enter in body & sick us.

3 = Father of Communicable disease in 1546.
→ These are those disease which spread through communication.

4 = Father of Microbiology is Louis Pasteur.
→ Introduced sterilization technique.
→ Pasteurisation of Milk.
→ In milk, all bacteria are kill 62.8°C on 30min.

5 = Father of Antiseptic surgery is Lord Joseph Lister
→ He instructed surgeons under his responsibility to wear clean gloves & wash their hand before & after operation with 5% carbolic acid solution.

Mine
Date : / /20
Page :

• Scope & ~~gi~~ microbiology :

1- Medical → This is use in medical line.
→ Which deals with the diseases of human & animals.

2- Immunology → Study of the immune system that protects the body from pathogens.

3- Agricultural Microorganism → Impact of microorganisms on agriculture.

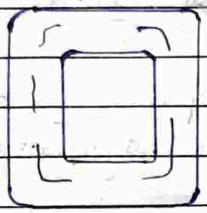
4- Industrial Microbiology → It is using microorganism to make products such as antibiotics, vaccines, steroids, alcohols etc.

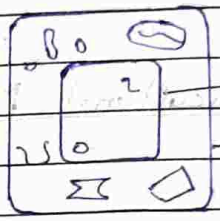
5- Food & Diary microbiology → It is use microorganism to make food such as cheeses, yogurt, pickles, beer, etc.

Q-2- Differentiate b/w Prokaryotes & Eukaryotes.

Prokaryote	Eukaryotes
Ans = These are those cells in which cell membrane are developed but nucleus membrane does not developed, also some organelles not developed.	There are those cells in which cell membrane, nucleus, their organelles are fully developed.
e.g. ~~Bake~~ Bateria & Protozoa etc.	e.g. Animal, plant, fungi, Algae, protist cells etc.

Ribosomes are small.	Ribosomes are large.
Growth rate are faster.	Growth rate are slower.
Plasma membrane are present.	Plasma membrane are present.
Cytoplasm are present.	Cytoplasm are present.





Q-3 → Write in details about isolation of pure cultures?

Ans → **Isolation of Pure Cultures** → A culture media, which contain more than one kind of microorganism is called a mixed culture media.

→ A culture that contain only one kind of microorganism is called a pure culture.

→ Isolation is the separation of a particular microorganism form the mixed culture.

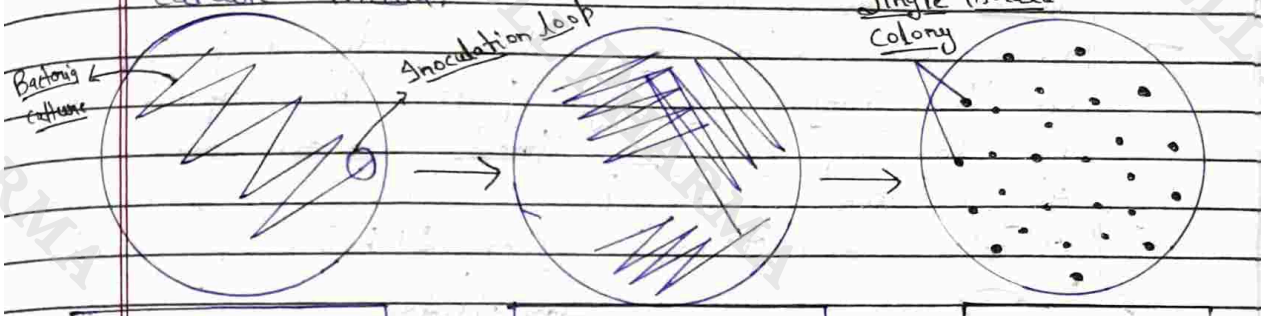
→ It is used to study about specially one species & for growth of any one species in their own pure culture.

→ The most commonly used method for pure culture:

1 → Streak plate technique.
2 → Pour plate technique.
3 → Spread plate technique.
4 → Serial dilution technique.

① → Streak Plate technique → In this technique, the bacteria is isolated from mixed culture media by applying streak on the surface of culture media with the help of inoculating loop.

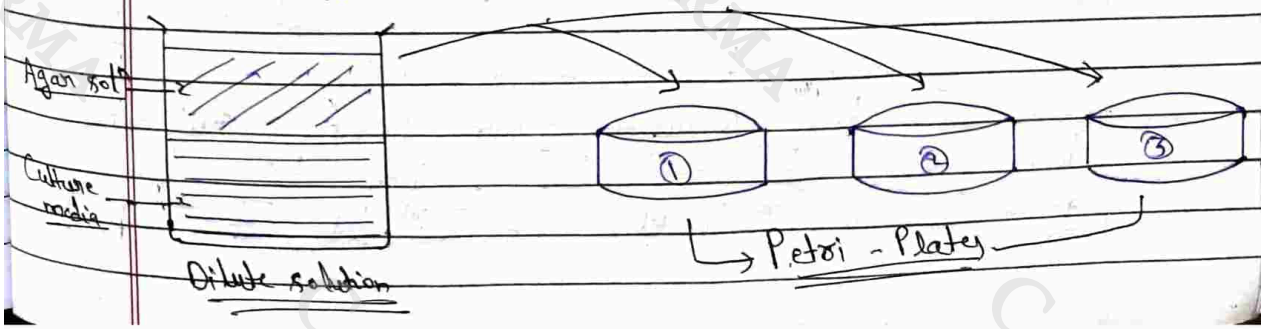
→ Firstly inoculation loop is heated for sterilization free from media then streak on the surface of the mixed culture & then it streak on new sterile culture media.



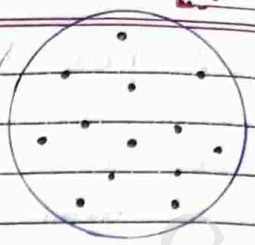
- Using a loop to streak plate.
- A properly streak plate
- Plate showing colony after inoculation

② → Pour Plate technique → In this method, dilute agar solution with water is mixed in the mixed culture media, & dilute the mixed culture media.

→ Now, these dilute culture media after proper mixing it transferred into petri plates.



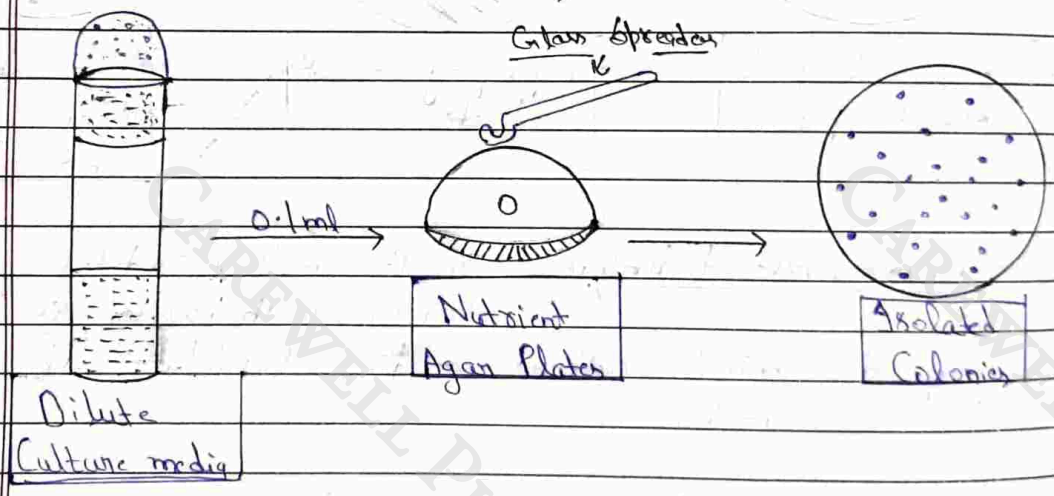
→ The colonies of similar shape, size & colour will be visible.



③ → Spread Plate technique → In this technique, a mixed culture media is diluted with some sterile water or saline solution.

Saline → [Salt + water]

→ Now take 0.1ml of culture media drop & placed on the surface of agar plate, then spread it with the help of glass spreader, on all over the surface, then allow bacteria to grow & isolated colonies are observed after 24 hours & counted.



④ → Serial Dilution technique → In this technique, take 5-7 test tube with filled 9ml of sterile distilled water solution.

Now take 1ml solution from original suspension & diluted it in Ist test tube, then take 1ml from Ist tube & diluted it in IInd tube. Then take from IInd,

Repeat this process until all test tube should be diluted.

1ml 1ml 1ml 1ml 1ml

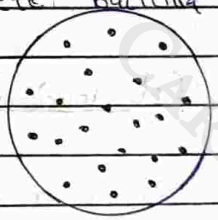
Original Suspension

SERIAL DILUTION

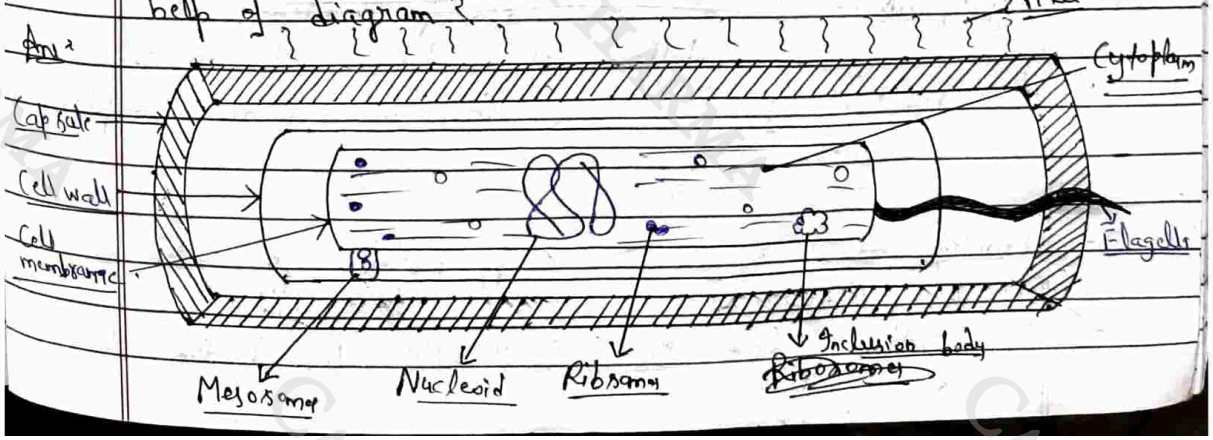
After dilute all tubes, take 1ml of each test tube & pour into nutrient agar plates.
As no. of test tube increase, the conc. of bacteria is decreases & the dilution is increases.

This method is used for actinomycete bacteria (soln.)

Take 1ml of from each & take it on separate nutrient agar plate.



Describe the structure of bacteria in details with the help of diagram?



Mine
Date : / /20
Page :

Bacteria → It forms a large group of unicellular prokaryotic that do not contain nucleus & other membrane organelles.

- **Structure of Bacteria** → On Ultra-microscopic View it look like as small capsule.
- **Parts of Bacteria**: There are various parts of bacteria.

1→ **Capsule** → It is outermost thick & slippery structure & flexible.
→ It is identity of bacteria & decide its ~~viral~~ virulence
→ It gives protection to the bacteria, ~~its virulence shape & size to bacteria~~, also give shape & size to bacteria, also prevent Phagocytosis.

2→ **Cell wall** → It is also thick structure, it is made up of Peptidoglycon layer.

3→ **Cell membrane** → It is thin layer in prokaryotic cells or bacteria. compared by cell wall.
→ It is made up with phospholipid bilayer.
→ It act as semi-permeable membrane.

4→ **Cytoplasm** → ~~It is less develop nucleus.~~
The cytoplasm of bacterial cells is where the functions for cell growth, metabolism & replication are carried out.

5→ **Nucleoid** → It is less develop nucleus is called as nucleoid.
→ It contain 60% DNA, 30% RNA, 10% Protein etc.

6→ Inclusion body → It stored food or food of bacteria is stored in the form of the inclusion body.

7→ Ribosomes → It helps in protein synthesis in the bacteria.
→ In bacteria, 70s types of ribosomes present.

8→ Flagellum → It is 15-20 nm long thick hair like structure & 2-5 nm diameter.
→ It work like as antenna, which gives signal to bacteria for food, dangers, locomotion etc.
→ It is made up by flagellin protein.

9→ Mesosomes → It is a slightly inside in cell wall.
→ It is extra chromosomal DNA.
→ It helps in cellular respiration.

10→ Pilli → It is a small thin 8-10 nm hair like structure.
→ It helps in attachment of bacteria with other bacteria.
→ It also helpful in transfer in genetic material.

Q-5→ Write a note on principle & application of Electron microscopy or phase contrast microscopy.

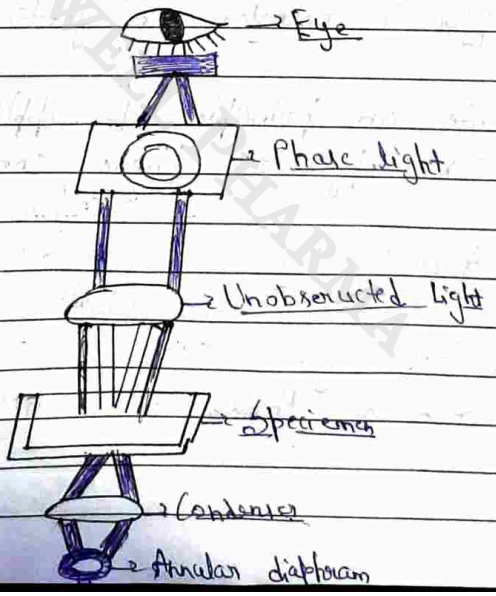
Ans→ Microscope → It is defined as it enlarge objects or microorganism, which we cannot see by our naked eye.
→ This phenomenon is known as Microscopy.

Mine
Date : / /20
Page :

Phase Constast Microscopy – There are the discovered by Friz Zernike in 1953.

→ It produces high-constrast images of transport specimens.
→ The phase plate is special optical disc located in the real focal plane of the objective, which retard the direct rays. & help to constrast the image.

– **Principle :**
→ When light passes, condenser ~~annulus~~ annulus modifies the light beam.
→ Then, when sample [specimen] is inserted it scattered parts of light which is then refocussed to the detector [image formed where].
→ To distinguish direct light from the scattered by the sample, a phase plate is inserted.
→ Light cross a thicker part of the plate, this shift its phase.
→ Then, this scattered light interferes with direct light which create a phase contrast.
→ This allow to see high constrast images of transport specimens, even no need to color them.



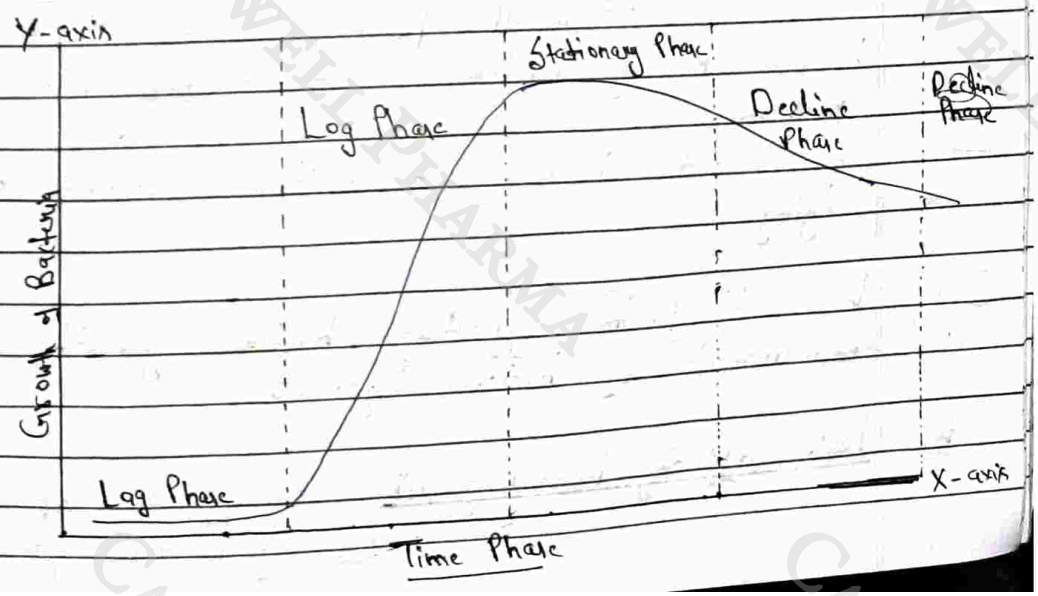
Application :

→ It is most commonly used to provide contrast of transparent specimens such as living cells or small organisms.
→ It useful in observing cells cultured in vitro during mitosis.
→ It is by far the most frequently used method in biological light microscopy.
→ It is an established microscopy technique in cell culture & live cell imaging.

Q-6→ Write a note on Growth Curve. ?

Ans→ When a graph plot b/w the growth of the bacteria & time phase is called growth curve.
The resulting curve has four phases are :

1→ Lag Phase.
2→ Log Phase.
3→ Stationary Phase.
4→ Decline Phase.



Mine
Date : / /20
Page :

(1) → Lag Phase → In this phase, their are no growth of bacteria, because culture media is just prepared, so upto 2 to 3 hours their are no growth of bacteria, only nutrition medium present.

(2) → Log Phase → In this phase, their are maximum growth of bacteria, because after the generation of the bacteria, they reproduce in faster rate & bacteria is increases, & their are nutrition are more than the bacteria, so bacteria grow maximum amount.

→ It is also known as Exponential Phase.

(3) → Stationary Phase → In this phase, growth of bacteria stop & it may be stationary, because no. of birth of bacteria is equal to the no. of death of bacteria.

→ It is just happend due to lack to nutrition medium.

(4) → Decline Phase → It is also known as Death Phase.
→ In this phase, bacteria start dying, because no. of bacteria is more than nutrition medium.

→ So due to lack of nutritions, bacteria start dying.

Q-7 → Write a factor affecting bacterial growth & measurement of bacterial growth?

Ans → Factor affecting bacterial growth → Some of the important factors affecting bacterial growth are:

Mine
Date : / /20
Page :

→ Nutrition concentration.
→ Temperature.
→ Gaseous concentration
→ pH.
→ Ions & Salt concentration.
→ Available water.

• Measurement of Bacterial Growth :→ It is calculated in two terms:

1→ Total count
2→ Viable count

(1)→ Total Count → In this measurment, count all bacteria (either it live or died).
→ It is also called direct method, because in which we directly count the no. of colonies of bacteria by using microscopes.

• There we count two methods following :
1→ Counting chamber method.
2→ Electron counter method.

(1)→ Counting Chamber method → In this method, firstly we required colonies counting chamber in which, we pass the cultures & one lens is upper side from where we watch the bacteria.
→ Now haemacytometer, it is look like seive but ~~rangu~~ rectangular shape, in which size of one hole is $1mm^2$, in which only one colony can identify.
→ Now, petri-plates placed inside the counting chamber, which containing culture media & then haemacyto-meter is fixed.

Mine
Date : / /20
Page :

→ When culture media passed, bacteria is colony is shown in the block of haemocytometer, which can easily counted, & then no. of bacteria is calculated.

(iii)→ Electron counter method → It used for measurement is coulter counter.

→ In this, bacterial suspension is passed through a capillary tube, diameter of tube is so microscopic that it allow only one cell to pass at a time.

→ When bacteria is passed b/w this, current pass & voltage generated & ~~are~~ one peak is formed in chart.

(2) Viable Count → In this measurement, we have to count only those bacteria which are alive, also grow regularly.

→ It is also called indirect method because in which we also count bacteria indirectly.

• There we count by two methods following:

i)→ Plate count method.

ii)→ Membrane filter count method.

(i)→ Plate count method → It measure the cell density & providing ~~information~~ information regarding the living bacteria only [viable].

→ In this method, culture media soln [in which we have to count bacteria] is diluted several times. [Same as serial dilution]., which we have done ~~to~~ in serial dilution technique.

→ After dilution take 1ml solution from each that test tube & put it on agar plate individually.

Mine
Date : / /20
Page :

→ Serial dilution helps to achieved 25 to 250 colonies in a single plate.
→ After incubated, the no. of colonies can be seen.

(iii) → Membrane filter method → In this method, we take membrane filter, which does not allow to pass the bacteria from membrane.
→ In which ~~the~~ we also mix the fluorescent dye, which attached with bacteria is colony, so we can easily identified bacteria.
→ When culture media passes though membrane filter, all the material content of culture media passes down & bacteria are separated on membrane filter, which we further collected in a new fresh culture media.

Q-8 → Describe in details about Culture media.?
Ans → In which, bacteria is birth or grow on culture media. Cultural media contain all essential material which is required for growth & development so, it invite bacteria for grow.

Culture Media

- Based on consistency
  - Solid
  - Semi-solid
  - Liquid
- Based on composition
  - Synthetic
  - Non-Synthetic
- Based on application
  - Basic C. media.
  - Enriched C. "
  - Selective C. "
  - Enrichment C. "
  - Differential C. "
  - Transport C. "
  - Anaerobic C. "
  - Assay C. media.

Mine
Date : / /20
Page :

1→ Based on Consistency → It is of three types are :
So, bacteria needs different media.
→ & , Agar is much more important for this.
→ Agar → It's a solidifying agent.

→ Solid → Agar [1·5 to 2%] . e.g, Nutrient Agar.
→ Semi-solid → Agar [less than 0·5%] . e.g, Nutrient broth.
→ liquid → Agar [Absent] . e.g, Fluid thioglycollate broth

2→ Based on Composition → It is depend upon the quality or quantity of material which is used to prepare culture media.
In which, material is used in this, : Energy source, Carbon source, Nitrogen source, Water growth factors & Salts etc.

→ Synthetic → [Highly pure], in which we know the composition of ingredients.
e.g, Peptone water. [Peptone 1% + NaCl in water 0·5%].

→ Non-Synthetic → [Impure] in which we don't know about the composition of ingredients.
e.g, Yeast extract broth etc.

3→ Based on Application → In which, we create culture media according to need of bacteria, on bases of application of bacteria.

(i)→ Basic culture media → It is applicable for all bacteria, because it contain all types of essential material, so in which all types of bacteria grow.

(ii) → Enriched culture media → It is for those bacteria, which needs extra nutritional material.

e.g. Blood, Serum [extra material for extra growth].

(iii) → Enrichment Culture media → It is ~~deo~~ designed for some specific type bacteria & in this media, antibiotic is used for reduce unwanted bacteria. So, that special type of bacteria grow easily.

(iv) → Selective culture media → These are ~~sp~~ specially designed culture media made for special or any one bacteria.

(v) → Differential culture media → It is a liquid medium. → In which metabolic dye is used to identify the different colonies by different color.

e.g. Blood Agar.

(vi) → Transport media → It is when culture media is made for long time or not from frequently. & it is present from dying.

→ It is used because in culture media bacteria is died in around 7 days, but if we need this after 15 to 20 days or after many days. So, we prepare transport culture media.

(vii) :- Anaerobic culture media :- It is used for those bacteria which does not required oxygen & in which we use Hemin or Vit. K & bacteria will easily grow.

e.g. Robertson's cooked meat medium.

(viii) :- Assay culture medium :- This culture media is used for study purpose, in which we have to know about the conc. of material which is used to make culture media.

→ It is perfect, or not & It's right, or not.

Unit :- 2

Q-1 :- Write in details about staining ?

Ans :- Staining :- It is a technique, in which we identify the bacteria, by using different technique. is called staining.

Staining
- Simple staining
- Differential staining
  - Gram staining
    - Gram positive
    - Gram negative
  - Acid-fast staining

• Simple staining :- In this staining, we observe the morphological characteristics [shape & size] of bacteria.

Mine
Date : / /20
Page :

→ In which, we use single stain dye such as crystal violet, safranine, methylene blue, malachite green.

- Principle → Firstly develop the -ve charge on the surface of the bacteria, so when we add +ve charge dye on bacteria, it will attached easily [-ve attract +ve charge & attached easily].
→ When dye attached on the surface of bacteria, bacteria will visible easily in also light background.
→ -Ve charge is develop on the surface of the bacteria by releasing H^+ ion or adding OH^- ion.

- Procedure :

→ Firstly take, glass slide, coven slip, inoculation loop, culture media, microscope.
→ Then, wash all these equipment with ethanol solution, after drying put under flamming for sterilization.
→ Now, take inoculation loop, streak into culture media, in which bacteria attached on loop, then streak inoculation loop on glass slide.
→ Now, add some drop of any indicator on surface of the slide.
→ Allow slide for drying, then wash the slide under tap water for remove excess stain.
→ Now, wipe the below surface of slide with tissue paper.

→ Then, put the cover slip over the surface of slide, where bacteria stained, then placed slide on the surface of microscope.
→ Now, see bacteria in microscope & observe the bacteria.
→ The blue coloured spherical shape or rod shape bacteria is seen.

• Gram Staining → In this staining, we identified that, bacteria is gram positive or gram negative.
→ This staining technique was developed by Han's christian in 1884.
→ In this, we use double or more dye at a time.
→ On the basis of their structure, bacteria is classified into two category:
* Gram positive & Gram negative.

- Procedure:

→ Firstly take glass slide, coven slip, inoculation loop, culture media, microscope & ethanol solution.
→ Now, wash all these equipments with ethanol soln, then, allow for drying, after drying put all the these under flamming for complete sterilization.
→ Now, take inoculation loop, streak into culture media in which bacteria attached on loop, then streak inoculation loop on glass slide.
→ Now, add some drops of crystal violet dye as an indicator & allow to dry.

→ Now, add gram iodine solution, which fix the crystal violet dye with cell wall.
→ Now, wash the slide with ethanol & Acetone soln.
→ Gram negative cell was has [8 to 12 nm] thin, so on washing crystal violet so detached from gram negative bacteria, but gram positive cell wall has [20 to 30 nm] thick, so washing crystal violet not detached & continously attached on surface of the bacteria.
→ Now, add few drops of Safranine dye as an indicator on the surface of slide & allow to dry.
→ Then observe it on microscope.

- Observation:

→ If purple/blue color obtained then bacteria is gram positive.
→ If red/pink color obtained then bacteria is gram negative.

• Acid-Fast Staining → It is also known as Ziehl-Neelsen staining, discovered by scientist Paul Ehrlich in 1883.
→ In this staining, use two or more dye.
→ It is used for those microorganism, which is wax like & impermeable cell wall [don't have cell wall].
→ These microorganism does not identified by gram staining, so used acid-fast staining, because it does not have cell wall.

Mine
Date : / /20
Page :

- Principle :

→ It is used to identify that bacteria is Acid-fast or Non-acid fast organism.
→ When we add carbal fuchsin dye as an indicator, it react with inside material of the bacteria in cytoplasm which is acidic & give red color to bacteria.
→ On the washing alcohol acid-fast some red color but non-acid fast decolarized

- Procedure:

→ Firstly take glass slide, coven slip, inoculation loop, culture media, microscope.
→ Now, take inoculation loop, streak into culture media in which bacteria attached on loop, then streak inoculation loop on glass slide & make smear on it.
→ Now, add few carbal fuchsin dye [primary dye] on surface of bacteria & allow for dry.
→ Then wash it with the alcohol [decolourizer].
→ Then further add some methylene blue [secondary dye].
→ Again wash the slide with water, then dry & observe it by seeing it on microscope.

- Observation:

→ If bacteria give red/pink color it Acid-fast.
→ If bacteria give blue/purple color it non-acid fast.

Mine
Date : / /20
Page :

Q-2. Describe the process the Sterilization by Autoclaving?

Ans: Sterilization → It is a process in which remove or kills of all types of microorganism from the surface of all non-living or living things.

- **AUTO CLAVE:**

→ It is also known as Steam sterilizer that is commonly use in ~~heat~~ healthcare facilities & industries for various purposes.

→ An autoclave is a machine that provides a physical method of sterilization by killing bacteria, viruses, & even spores present in the material put inside of the vessel using steam under pressure.

→ The autoclave maintain a temperature of 121°C for at least 15 minutes.

- Principle :→ In which, saturated steam is produced under pressure.
So, pressure increase in a closed vessels increase temperature proportionally.
And, then high pressure is used to kill the microorganism.

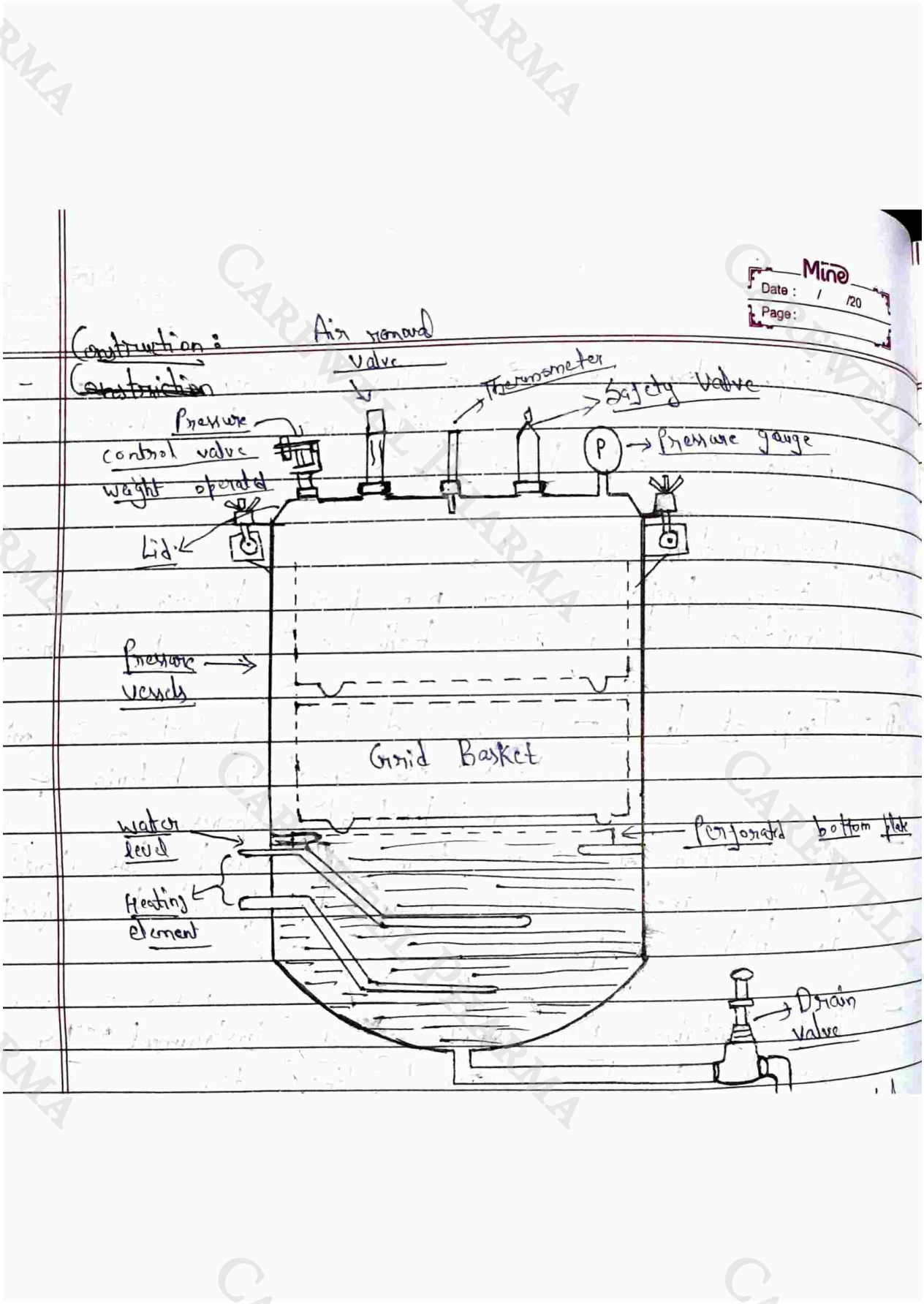
It is sufficient to kill the vegatative & spores of the microorganism.
So, it is the best/better & most widely method used for sterilization.

- Construction :

→ It consist of a verticle, cylinder of gun metal or stainless steel in a supporting frame or case.

→ The lid is fastened by screw clamps & rendered airlight by a asbestos gasket.

→ The autoclave has on its lid on upper side a discharge tap for air & steam, a pressure gauge & a safety valve that can be set to blow off at any desired pressure.



- Procedure → The water is filled in autoclave & material is to be sterilization is load inside autoclave.
- Now, maintain the temperature & pressure for sterilization, according to their need.

Temperature (°C)	~~Steam pressure~~ [lb/sq.inch]	Holding time [min]
115 to 118	10	30
121 to 124	15	15
126 to 129	20	10
135 to 138	30	3

- After put the material, lid is closed, discharge tap is opened & safety valve is adjusted to the required pressure.
- When air ~~bubbles~~ stop emitting from the discharge tap, it indicates all the air removed from inside the autoclave.

→ Then, close the discharge tap. Now, steam pressure rises inside & when it reaches the desired set level, safety valve opens & excess steam escaps.
→ After the holding time, the heating is stopped & allowed to cool the autoclave.
→ Now, air is allow to remove from the autoclave by open discharge tap.
→ Now, finally lid is opened & the sterilized articles [material] are removed.

- Application → It is used for sterilization Bacteriological media, heat stables liquid, saline soln, heat resistant equipment & instrumentals, glasswear, filters, rubber products etc.

- Advantages :

→ It is very effective.
→ It gives provide greater lethal action of moist heat.
→ It is very quicker in heating up the exposed articles [material].

- Disadvantages :

→ It is not suitable for heat labile substance, which cannot with stand heating at 115°C & above.
→ It is not suitable for anhydrous material such as powders, oils, fats .etc.

Mine
Date : / /20
Page :

Q-3 : Different b/w Gram positive & Gram negative staining?

Ans :

Gram Positive	Gram Negative
→ Peptidoglycan's ~~single~~ multiple layer present.	Peptidoglycan's single layer present.
→ It is outer membrane is absent	It is outer membrane is present.
→ Cell wall 20 to 30 nm thick & single layer	Cell wall 8 to 12 nm thin & double layers.
→ After staining it gives purple/blue color.	After staining it gives red/pink color.
e.g., Streptococcus, bacillus.	e.g., Escherencheia coli etc.

Unit - III

Q-1 : Write in details about Virus or Fungi such as morphology, classification, reproduction/replication, cultivation?

Ans : It is FUNGI

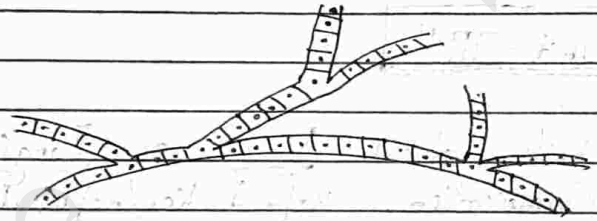
→ It is a group of Eukaryotic micro-organisms.
→ All fungi are chemoheterotrophs, requiring organic compound for energy & carbon.
→ Fungi are aerobic in nature [except some yeast], which are facultative anaerobic].
→ They may be unicellular or multicellular.
• It is a branch of science, which deals with the study of fungi is called Mycology.

Mine
Date : / /20
Page :

- Morphology :→ It can be divided into four classes.

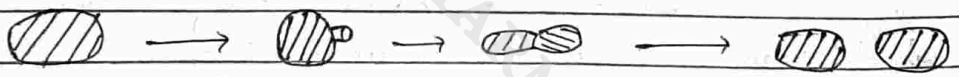
(i)→ Moulds → It is also called Fleshy fungi.
→ It contain multiple identical nuclei.
→ It forms fuzzy appearance of black, green, brown, orange, pink colonies on the surface of media.
→ These are strictly aerobic & optimum temperature for growth is 22° to 28°C.
→ It reproduce through small pores, which can be either sexual or asexual.

eg, Penicillium notatum, Aspergillus nigar etc.



Mould

(ii)→ Yeasts → It is round & oval in shape or elongated unicellular fungi.
→ Most of them reproduce by an asexual process called Budding.



Stages of Budding

→ It forms white, circular, smooth, creamy colonies on the surface of media.

Mine
Date : / /20
Page :

→ They are aerobic but some yeasts are facultative anaerobic.
→ The optimum temp. for growth of yeast is 32-37°C.
e.g. Cryptococcus neoformans etc.

(iii) Yeast like fungi → In some yeast like Candida albicans, the bud remains attached to the mother cell & elongated, followed by repeated budding, forming chains of elongated cells known as Pseudohyphae.

→ It forms moist creamy coloured colonies on the surface of solid media.

(iv) → Dimorphic Fungi:

→ Some fungi, mainly pathogenic species exhibit dimorphism i.e., two forms of growth.
→ Fungi can grow either as a mould or as a yeast.
→ It is temp. dependent so, at 25°C it shows mould like growth but at 37°C it shows yeast like growth.
e.g. ~~Hist~~ Histoplasm capsulatum etc.

– Classification: It can be divided into four parts:

(i) → Zygomycetes → They are fungi having non-septate hyphae [lower fungi].
→ It form asexual spores contained within sac like structure called Sporangia.
e.g. Mucor.

(ii) → Ascomycetes → It forms Sexual spores within a Sac & are called Ascospores.
→ Sac is called ascus.
→ They include both yeasts & moulds.
e.g., Histoplasma.

(iii) → Basidiomycetes → It reproduce sexually & form septate hyphae.
→ These are borne at the tip of the basidium.
e.g., Mushrooms etc.

(iv) → Fungi imperfecti → It is also called as Hyphomycetes or Deuteromycetes.
→ They consist of group of fungi whose Sexual phases are not identified.
→ They are grow as molds as well as yeasts.
e.g., Candida albicans etc.

– Reproduction/Replication of fungi: They reproduce by both Sexually & asexually

(i) → Sexually → In this reproduction, Sexual spores is formed by fusion of cells & these are produced by meiosis.
→ Two different cells i.e. male & female cells. fused called plasmogamy then further fusion of two nuclei called Karyogamy.
→ As a result zygote is formed, which further Sexual spores.
→ Sexual spores are like as Basidiospores, Ascospores, Zygospores.

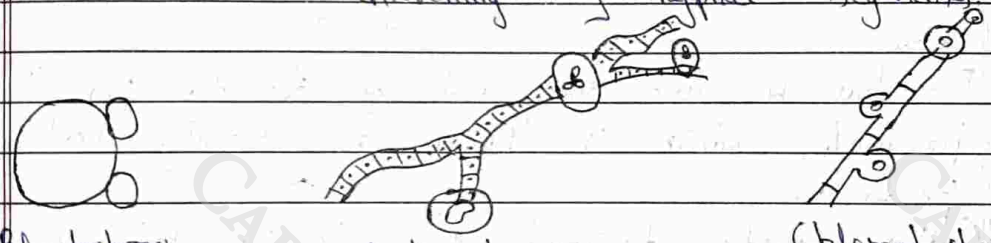
(ii) → Asexually → In this reproduction, asexual spores are produced by mitosis.
These are divided into two types are :
a) → Vegatative spores.
b) → Aerial spores.

(a) → Vegetative spores → It can be divided into three types.

→ Blastospores → These are formed by budding from parent cells, as in yeasts.

→ Arthrospores → These are formed by segmentation & condensation of hyphae.

→ Chlomydospores → These are thick walled resting spores develop by rounding up & thickening of hyphal segments.



Blastospores Arthrospores Chlomydospores

(ii) → Aerial spores :

→ Conidiospores → They spores borne extesenally on sides or tips of hyphae are called conidiospores. or Conidia.

Cultivation of fungi :→ In cultivation of fungi, we have to identify fungi, then separate & grow fungi.

→ Sabouraud agar is a type of agar growth medium containing peptones. It's used to cultivate many types of fungi & also grow.
→ It has utility for research & clinical care.
→ It is developed by Raymond Sabouraud in 1892.
→ The standard temp. for incubation of fungi is 30°C in humidified environment for 21 days.
→ After 21 days colonies of fungi on culture media

Q-2→ Briefly explain the method for carrying out the sterility test on pharmaceutical products ?

Ans→ After the sterilization process of any product, material we have to check whether the product that we have sterilized is truly free of bacteria or not.

Mine
Date : / /20
Page :

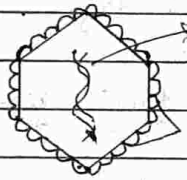
VIRUS

→ Viruses are the smallest infectious agent, these are very small, so we can watch it with the help of electron microscope [ultra microscope].

→ They are about 10-100 times smaller than most of the bacteria.

→ They're composed of genetic material DNA or RNA [anyone] & protein.

– General Structure of Virus :



→ Nucleic acid (DNA or RNA) [anyone]
neither both.

Capsid (protein)
↓
highly specialized protein coat, that protect genetic material.

→ Viruses are obligate parasite, ie, totally depends on its host [they are incable of independent growth in artificial growth].

→ Viruses are non-living thing until they enter inside the living body [host]. So,
– Outside the living body (without host) → Non-living.
– Inside " " " (with host) → Living thing.

→ The complete infective form of a virus outside a host cell with a core of DNA or RNA & a capsid [protein]. is called Virions. → for complete structure of virus.

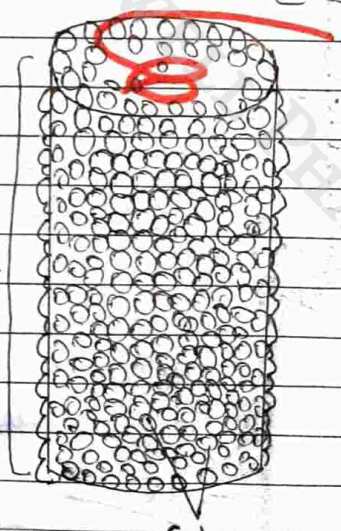
- Virology ↔ In which, we study about viruses.
- On the basis of their Morphology, Viruses divided into 3 types:

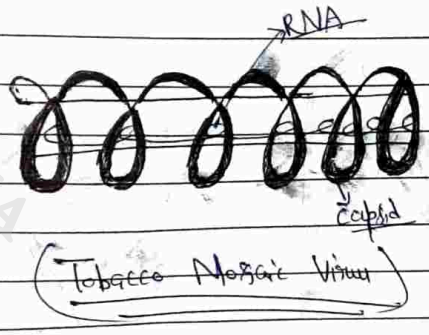
1- Helical Virus [Rod shaped viruses].
2- Polyhedral [Icosahedral] " ".
3- Complex virus.

1) Helical Viruses → They're rod-shaped like structure.
→ They have capsid made from over thousand molecules of single type of protein arranged in a helix.

→ The genetic material is generally single-stranged RNA [sometimes DNA] bound into the protein helix by interaction b/w the negatively charged nucleic acid & +ve charge on protein.

e.g., Tobacco mosaic virus [TMV]., Rabies Viruses, etc.





Tobacco Mosaic Virus

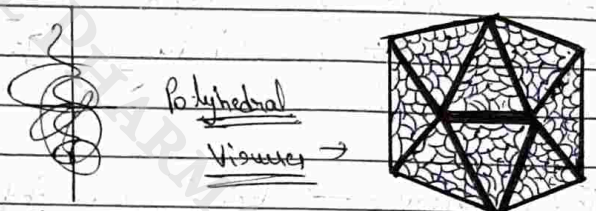
2)- Polyhedral [Icosahedral] Viruses → They're round shaped.

→ It also called as spherical Viruses.

→ Icosahedral means, it have 20 sided/ faces & may be more than 20 [polyhedral].

→ It gives roughly spherical appearance.

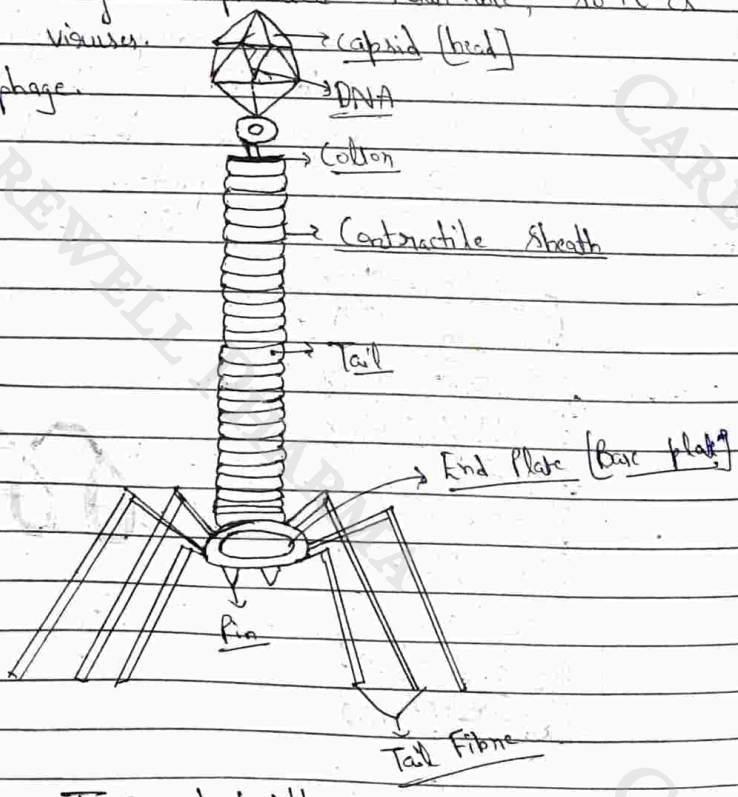
e.g. Adenovirus, Poliovirus, etc.



3)- Complex Viruses → They're tad pole like structure i.e. [head & tail].

→ It have very complicated structure, so it is called complex viruses.

e.g. Bacteriophage.



T- even bacteriophage

Mine
Date : / /20
Page :

- Classification → On the basis of host :
  - i) Animal Viruses.
  - ii) Plant Viruses.
  - iii) Bacterial Viruses. [Bacteriophages].

i) Animal Viruses → They parasite on animals & cause disease on them.
- They contain both DNA or RNA [anyone] as genetic material.
- They causes diseases → Polio, Hepatitis, AIDS or many more.

ii) Plant Viruses → They parasite on [infect] plants & cause disease on them.
- Plants may be potato, sugarcane, tobacco etc.
- They have only RNA.

Diseases → Tabacco Mosaic Virus [TMV] etc.

iii) Bacteriophages → The viruses infect on bacteria & cause disease on bacteria.
- They contain only DNA as genetic material.
- They may be infect E. coli bacteria, & it is called T even phages [T_2, T_4, T_6].

- Replication / Reproduction of Viruses :

For replication of virus host is necessary host may be bacteria, plants or an animals.
- When virus infect on host cell, a host cell is forced to rapidly produce thousands of identical copies of original virus.

=> Steps for Replication:

1) Attachment.
2) Penetration
3) Uncoating [Biosynthesis].
4) Assembly [Maturation].
5) Release.

1. Attachement → In this step, virus attached to the surface of host cell, they attached on specific receptor on plasma membrane of host cells.

2. Penetration → In this step, virus enter inside the host cells, [only DNA/RNA of virus enter or complete virus enter].
→ Entry of the virus.

3) Uncoating & Biosynthesis → In this step, viral capsid is removed by viral enzymes or host enzyme.
→ Due to capsid removed viral genomic nucleic acid release in host cells.

• Biosynthesis [Genome replication] → The replication mechanism depends on the viral genome
→ DNA viruses usually use host cell protein & enzymes to make additional DNA that is transcribed to messanger RNA, which in then used to direct protein synthesis.
→ RNA viruses usually use the RNA core as a template for synthesis of viral genomic RNA.

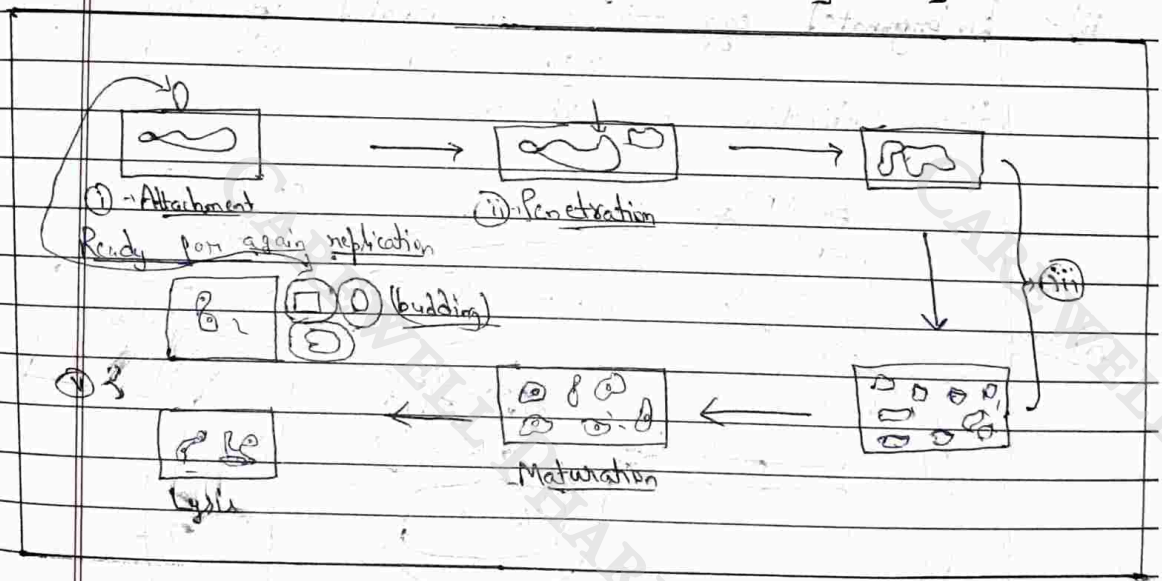
Mine
Date : / /20
Page :

4 - Assembly [Maturation] → After biosynthesis modified viral protein are packaged with newly replicated viral genome into new virions that are ready for release from the host cell.

5- Release → Two method for release :

(Rupture)
(i) Lysis → In this, viruses burst the cell membrane of host cell & release.
[death of host cell] [Cytolytic].

(ii) - Budding → In this viruses, make envelope from plasma membrane of host cell & release, does not harm host cell. [Influenza].



Mine
Date : / /20
Page :

Cultivation of Viruses :

Viruses are obligate parasites, so they can't grow on culture media.

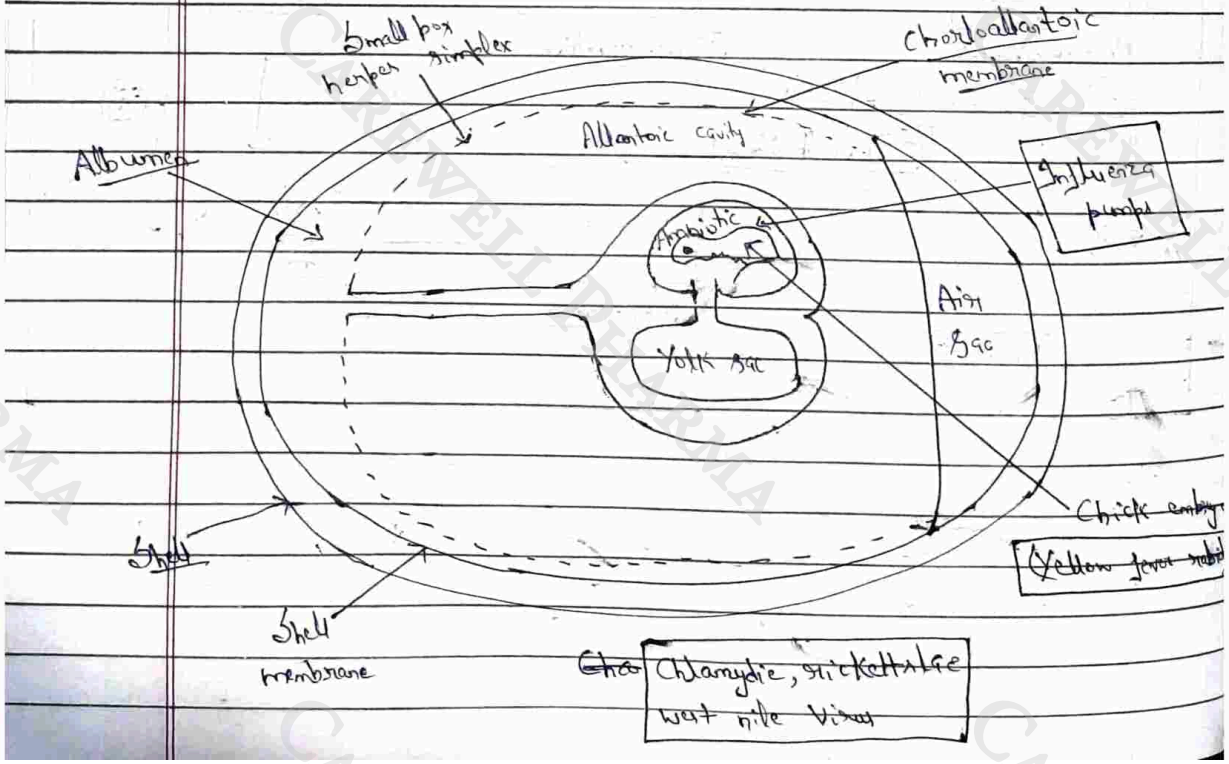
→ Cultivation of viruses are as follows :

i) → **Laboratory animals** → It's one of the oldest method for cultivation of viruses.

→ Animals use for this, such as rabbits, guinea pigs, mice (rat), ferret etc.

→ Animal inoculation is used for the study of pathogenesis, immune response & epidemiology.

ii) → **Embryonated eggs** → The embryonated eggs hen's egg for several sites for the cultivation of viruses.



Mine
Date : / /20
Page :

→ Fertile chicken eggs incubated for 5-12 days can be inoculated through the shell aseptically. The opening may be sealed with paraffin wax & the egg incubated at 36°C, for the time required for the growth of the viruses.

→ Viruses may kill the chick embryo & produce specific evidence of Viral activity.

→ These effect help in the identification of virus.

Q-2:- Briefly explain the method for carrying out the sterility test on pharmaceutical products ?

Ans:- After the sterilization process of any product, materials we have to check whether the product that we have sterilized is truly free of bacteria or not.

→ For this we use some test of sterility for that products, which is known as sterility testing of products.

- Culture media designed for sterility testing :

(i) :- Fluid thioglycollate → These are growth for anaerobic bacteria also for some aerobic bacteria.

(ii) :- Soyabean Casein Digest :- These are growth for the fungi & aerobic bacteria.

- Methods for Sterility test → Sterility test can be carried out by using the following methods :

1 :- Method - A
2 :- Method - B.

Mine
Date : / /20
Page :

① Method - A : It is also known as membrane filter.

- Products → An oil, ointment, soluble powder, liquid products etc.

- Procedure :

Sample
Dilute ↓
Aseptic filteration through membrane filter.
After filteration ↓
Cutting of filter paper in two parts.
↓
Place each part of filter paper in culture media separately for detection of aerobic/anaerobic bacteria or fungi.
↓

Culture media I st	&	Culture media II nd
on Fluid thioglycollate		on Soyabein Casein
↓		↓
30° to 35° C for 14 days		20° to 25° C for 14 days

Incubate & observe for any growth of bacteria.
↓↓
If microbial growth was observed, then sample failed in sterility testing.
Or
If microbial growth was not observed, then sample passed in sterility testing.

(2) → Method B : It is also known as Direct Inoculation

- Products → Aqueous soln, suspension, oily soluble products, surgical dressing, sterile devices etc.

- Procedure : Sample

↓

Direct addition of sample into the culture media separately for aerobic / anaerobic bacteria :

Culture media Ist or Fluid thioglycollate → 30-35°C for 14 days

Culture media IInd or Soyabin Casein → 20-25°C for 14 days

Incubate & observe for any bacterial growth

↓

If microbial growth was observed, then sample failed in sterility testing

Or

If microbial growth was not observed, then sample passed in sterility testing.

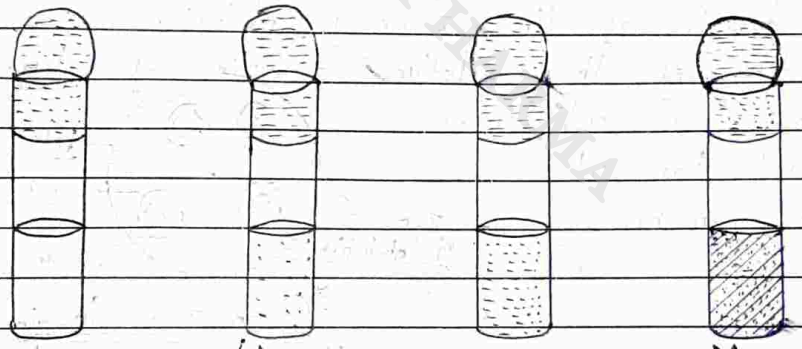
Q-3 → What are disinfectants ? Add a note on evaluation of disinfectants ?

Ans → Disinfectants → These are antimicrobial agents that are applied to non-living object to destroy microorganism & the process is known as disinfection

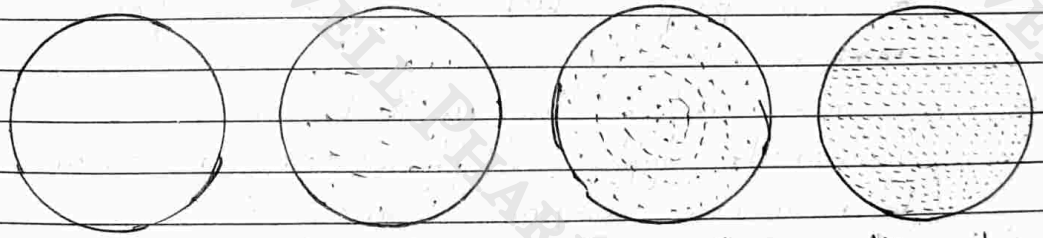
- Evaluation of Disinfectants:

1) Tube dilution & Agar plate method:

The chemical agent is incorporated into nutrient broth or agar medium & inoculated with the test microorganism.
These tubes are incubated at 30°-35°C for 2-3 days & then the results in observe.



A- Tube dilution Method



B- Agar plate dilution method

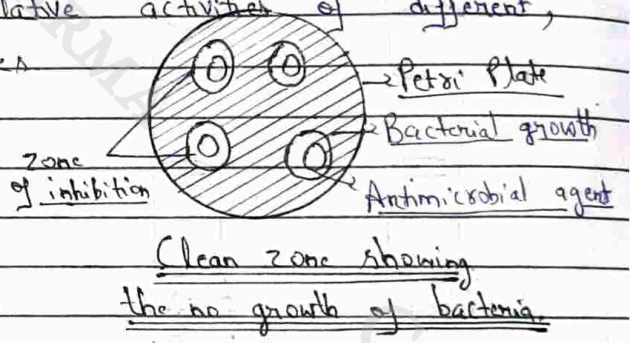
The results are recorded & the activity of the given disinfectants is compared as shows in figures as B.

(2) → **Cup-plate method** → In this method, the agar is melted & cooled at about 45°C, inoculated with the test microorganism & poured into a sterile petri-plate.

→ When the inoculated agar has solidified, holes about 9mm. in diameter are cut in the medium with a sterile cork borer.

→ The antimicrobial agent is directly placed in the holes, the zone of inhibition is observed after incubation at 30° to 35°C for 2-3 days.

→ The diameter of the zone of inhibition gives an indication of the relative activities of different, antimicrobial substances against the test microorganisms.



Clean zone showing the no growth of bacteria

(3) → **Phenol Coefficient method** → In this method, a test chemical is rated for its microbial property with reference to phenol under identical condition.

→ Organism used in the methods are: Salmonella typhi, Staphylococcus a aureus, Pseudomonas aroginosa are used.

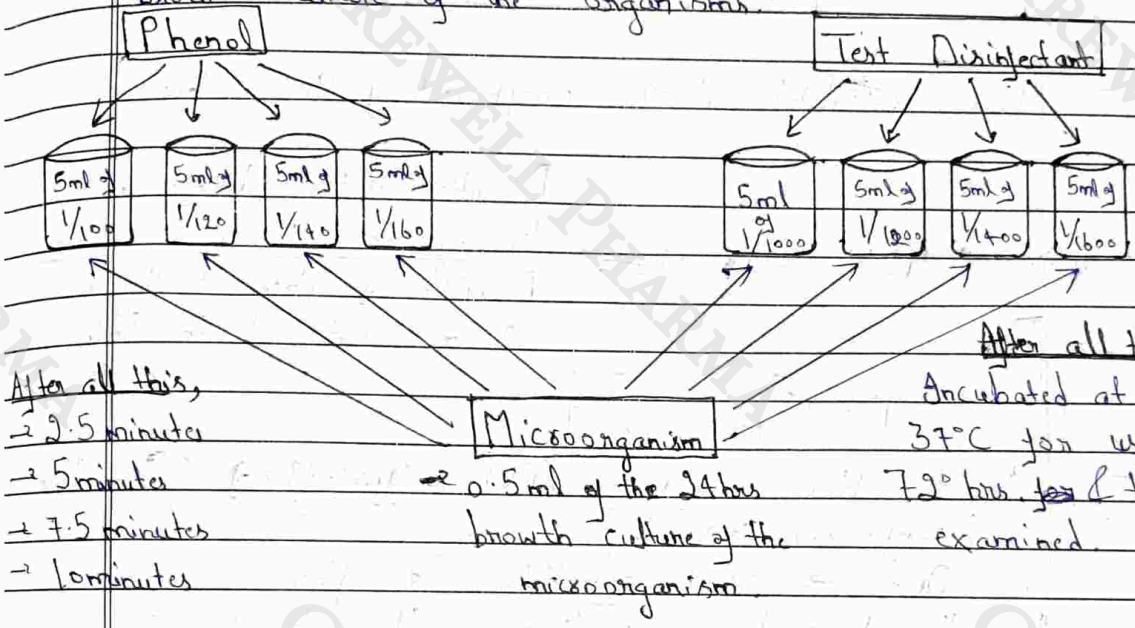
i) → Rideal - Walker Test.

ii) → Chick - Martin Test.

(i) → **Rbl Test** → The phenol coefficient of test disinfectants may be calculated by Rideal walker test. that are use Rideal walker broth & salmonella

Typhi as the sensitive microorganism.

Procedure → Different dilutions of the test disinfectants & phenol are prepared & 5 ml of each dilutions is inoculated with 0.5ml of the 24 hrs broth culture of the organisms.



After all this,
→ 2.5 minutes
→ 5 minutes
→ 7.5 minutes
→ 10minutes

After all this, Incubated at 37°C for us to 72 hrs & then examined.

→ After this, All tubes are placed in a water bath at 17.5°C.
→ Subcultures of each reaction mixture are taken & transferred to 5ml sterile broth after 2.5, 5min, 7.5 & 10minutes.
→ The broth tubes are incubated at 37°C for 48 to 72 hrs & are examined for the presence or absence of growth.

Disinfectant	Dilution	Time interval for sub-culture (min)			
		2.5	5:00	7.5	10:00
Test disinfectant	1:1000	+	−	−	−
	1:2000	+	+	−	−
	1:3000	+	+	+	−
	1:4000	+	+	+	+

Disinfectant	Dilution	Time interval for sub-culture (min)			
		2·5	5:00	7·5	10:00
Phenol	1:80	+	−	−	−
	1:100	+	+	−	−
	1:120	+	+	+	−
	1:140	+	+	+	+

→ Now, calculated R-W coefficient of the disinfectant.

R·W. coefficient ⟹ $\frac{\text{Dilution of test disinfectant. Killing in 7·5 but not 5 min}}{\text{Dilution of phenol Killing in 7·5 min but not in 5 min}}$

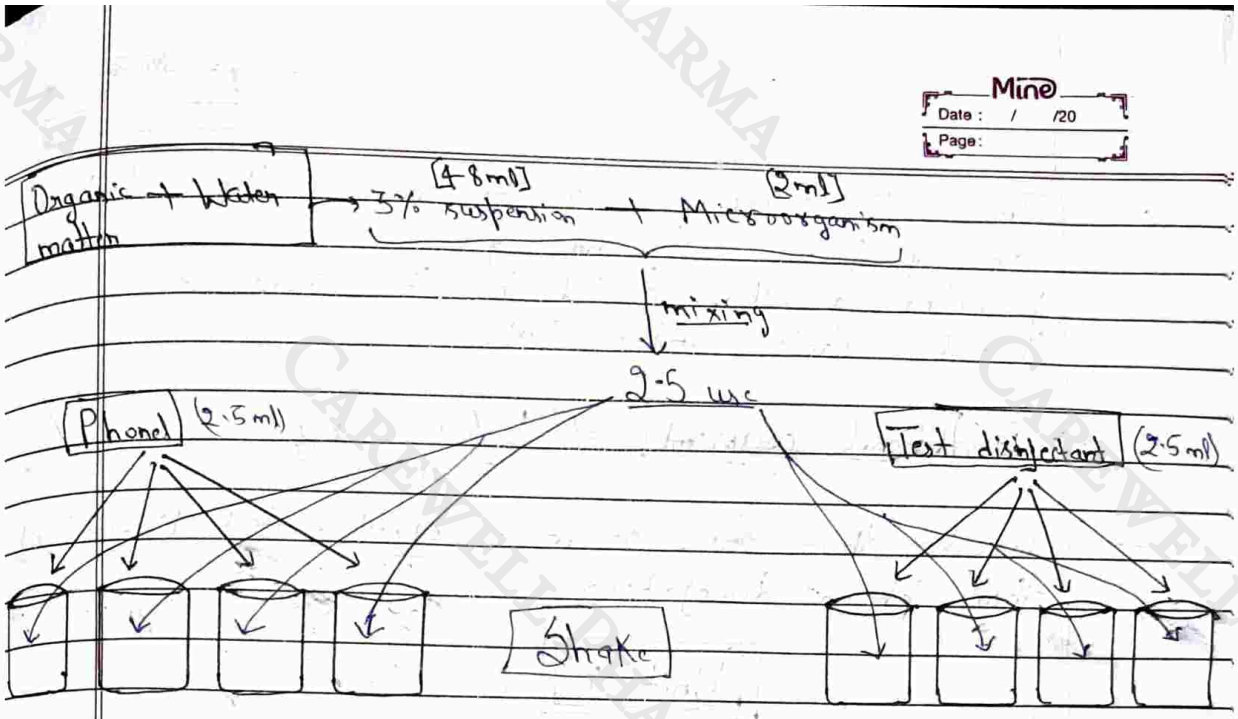
R·W. ⟹ $\frac{2000}{100} = 20$

→ If the phenol coefficient [RW coefficient] of given test disinfectant is:
One → Same effectivess as phenol
Less than 1 → Less effectiveness than phenol.
More than 1 → More effectiveness than phenol.

→ There is 20 times ~~perform~~ test disinfectant is 20 times more active than phenol.

(ii) → Chick Martin test → In this test, is done in controlled amount of organic matter in the form of standardization suspension of yeast cells.

→ In this test all the conditions like Rideal test.



→ Take one loopful of reaction mixture to 10ml recovery culture media & incubation on 37°C for 2days

• One loopful → [Phenol + microorganism]

[Test disinfectant + microorganism].

→ One loopful of reaction mixture is different-different conc. of phenol & test disinfectant.

→ Same R.M test but the temperature is 20°C & the exposure time is 30min.

Phenol	tube		Test disinfectant	tube	
%	①	②	%	①	②
2.0	−	−	0.47	−	−
1.80	−	−	0.41	−	−
1.62	+	−	0.37	+	+
1.45	+	+	0.33	+	+

$\frac{1.8+1.45}{2} = 1.625$ $\frac{0.41+0.37}{2} = 0.39$

→ The C.M coefficient is the mean of the highest conc. of phenol showing growth & lowest conc. preventing growth, divided by the same mean for the disinfectants under test.

∴ Ex: Phenol coefficient = $\frac{1.625}{0.39} = 4.16$

→ Greater than one, so more effective [test disinfectant] than phenol disinfectant.

Unit-4

Q-1→ Discuss the design & layout of an aseptic area?

Ans→ An aseptic area is a room, within a clean area designed, constructed, serviced & used with the intension of preventing microbial contamination of the products.

→ Aseptic technique are used to prevent the acess of microbial & particulate contamination into opthalmic & parental products.

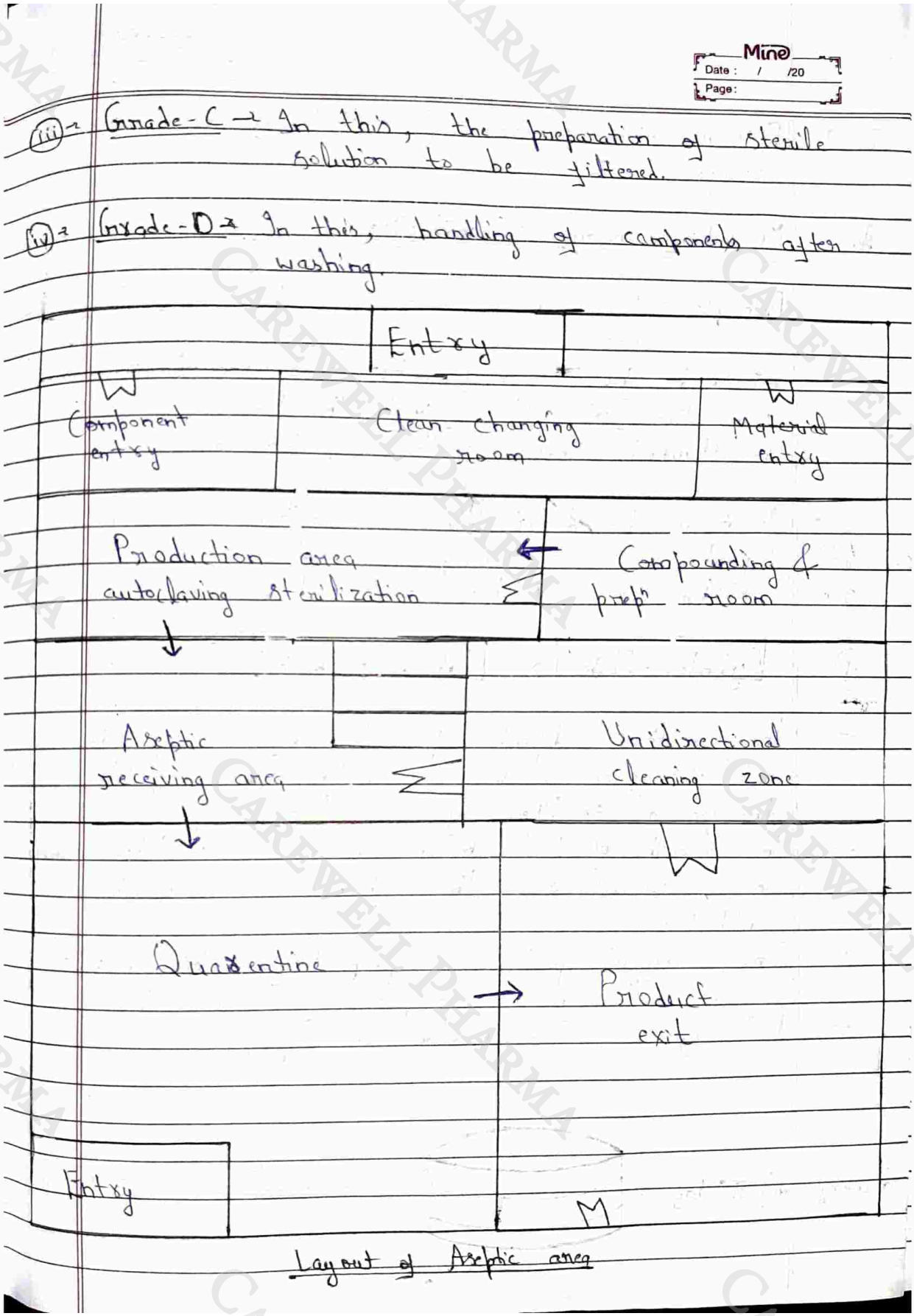
→ The aseptic area is divide into four main grades according to their operations:

(i)→ Grade-A → The local zone for high risk operations.
e.g. filling & making aseptic transfer.

(ii)→ Grade-B → Background area of Grade-A processes [aseptic preparation & filling].

(iii) :- Grade-C :- In this, the preparation of sterile solution to be filtered.

(iv) :- Grade-D :- In this, handling of components after washing.



Layout of Aseptic area

Q-2:- Discuss the principle & methods of microbiological assay?

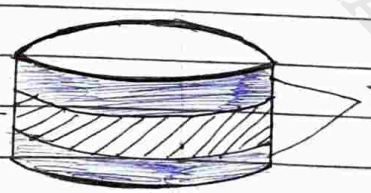
Ans:- **Microbial assay of antibiotics:**

- **Principle** :- It is a based upon a comparison of the inhibition of growth of micro-organism by measured the conc. of the antibiotics under examination [test antibiotics] with the known conc. of a standard known antibiotics preparation.

- It is can be divide into two methods are usually:
1:- Cylinder-plate or Cup-plate method.
2:- Turbidimetric or tube assay method.

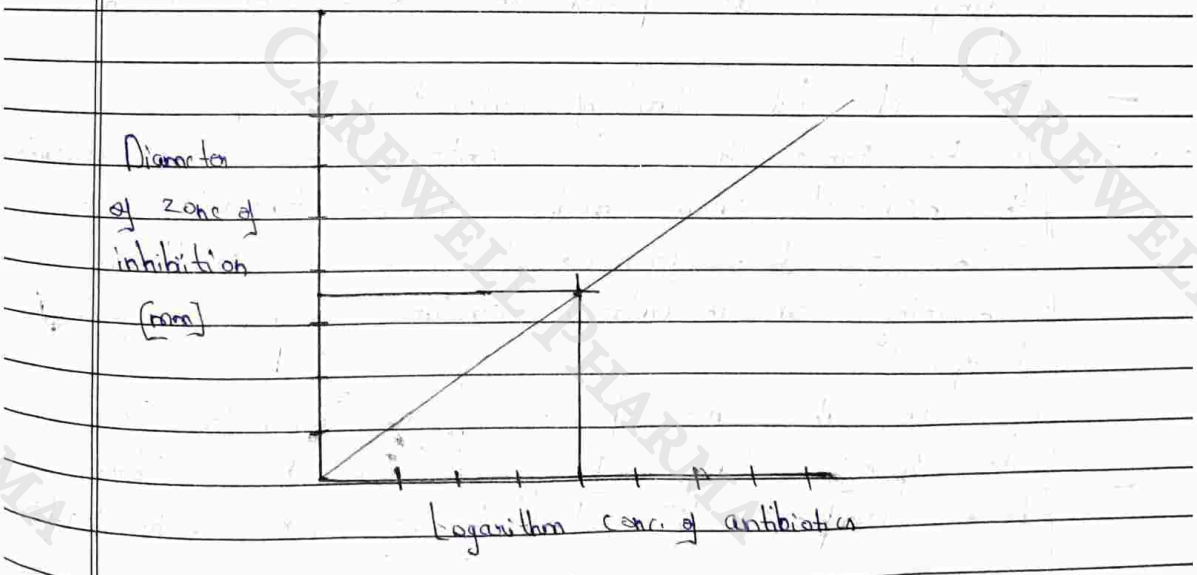
① :- **Cylinder-Plate or Cup-Plate method:**

→ This methods depends upon the diffusion of an antibiotic from a vertical cavity or a cyclinder, through a solidified agar layer in a petri-plates.
→ The growth of test microorganism is inhibited entirely in a circular area the cylinder soln of the antibiotics.
→ Three layered plates are prepared for this method:
- I^st & III^rd layer is prepared of agar medium.
- II^nd layer of agar medium was prepared.



Mine
Date : / /20
Page :

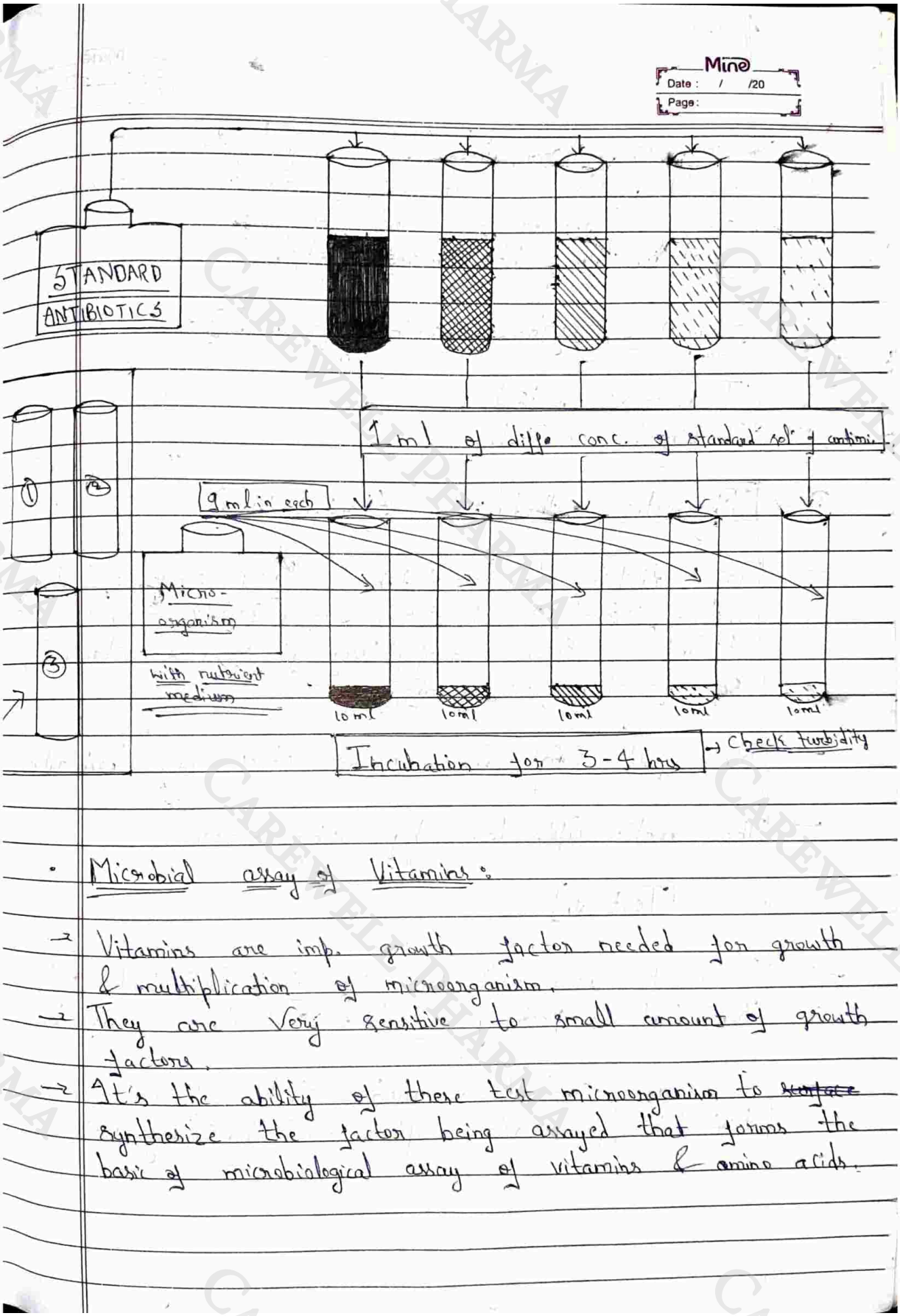
→ Now, the holes are made in agar media by using cork borer & antibiotics solution are filled in those holes with the help of micropipettes.
→ Solution of known conc. of the standard prep^n & the test antibiotics are prepared in appropriate soln.
→ These solution are added in sterile cavities prepared in agar medium separately.
→ The plates are left standing for 1 to 2 hrs at room temp. are 4° C.
→ All plates are than incubated for about 18-24 hrs at temp. [according to bacteria & antibiotics].
→ The diameter of the circular inhibition zones produced by standard & test antibiotics soln are accurately measured.
→ The graph which relates zone diameter to the logarithm of the conc. of antibiotics is plotted & the unknown concentrated of test antibiotics is ~~calcu~~ calculated.



Mine
Date : / /20
Page :

Turbidimetric method

- This method depends upon the growth of microbial culture in a uniform soln of the antibiotics in a fluid medium that is favourable to its rapid growth in the absence of the antibiotics.
- Here, turbity shows the microbial growth.
- It has a shorter incubation periods for the growth of test microorganism 4 to 5 hours.
- This method is not recommended for cloudy or turbid preparation.
- Five difference conc. of the standard soln are prepared by diluting the stock soln for making the standard.
- A median conc. is selected & the test sample of the antibiotics soln is adjusted by dilution to obtain approx this conc.
- 1 ml of each conc of the standard soln & of the sample soln are placed in each of the tubes in duplicate.
- To each tube, 9 ml of nutrient medium.
- At the same time, 3 control tubes, one containing the inculated culture media, second treated with 0.5 ml of dil. formaldehyde soln & third containing un-inoculated culture media are prepared.
- All the tubes are placed in an incubator at the specific temp. to approx 37°C for 3-4 hrs.
- Now compared the turbidity of test with standard



Microbial assay of Vitamins :

→ Vitamins are imp. growth factor needed for growth & multiplication of microorganism.

→ They are very sensitive to small amount of growth factors.

→ It's the ability of these test microorganism to ~~surface~~ synthesize the factor being assayed that forms the basic of microbiological assay of vitamins & amino acids.

→ Test microorganisms used for assaying the water soluble vitamins such as Vitamin B_{12}, Vitamins B_6 as test microorganism used such as: Lactobacillus leichamannii etc.

• Assay of Cyanocobalamin [Vitamin B_{12}]:-

i) → Titrimetric method.
ii) → Turbimetric method.

① → **Tibrimetric method** : (Standard bired)

→ Take ten clean test tube & fill 0ml, 0.5ml, 1.0ml, 1.5ml, 2.0ml, 2.5ml, 3.0ml, [illegible], 4.0ml, 4.5ml & 5.0ml respectively of standard vitamin B_{12} soln. [0.01 to 0.04 µg/ml].

→ Now add 5ml Basal media stock soln. (each tube).

→ Then make upto 10ml by using water.

[Test ↓]

→ Take 4 clean test tube & fill (add) 1ml, 2ml, 3ml, 4ml respectively of test soln to be assayed.

→ Now add 5ml Basal media stock soln to each test tube & make upto 10ml by using water.

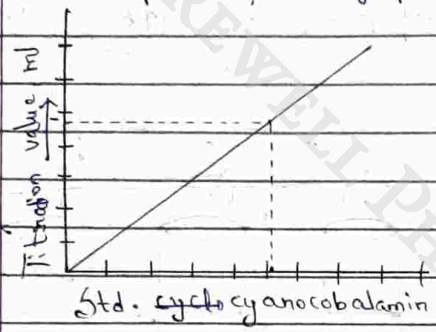
→ Now ~~sterilize~~ Sterilize all tubes [standard + test] in autoclave at 121°C for 5mint, then cool to all test tubes upto room temp & inoculate with one drop of inoculum [test microorganism lactobacillus].

→ Incubate the tubes for 64 to 72 hrs at temp within 30 - 37°C.

→ Now, titrate the content of each test tube [standard + test soln] with 0.05N NaOH by using 0.1% w/v bromothymol blue as an indicator or by electrometrically. (Convert to green color)

Mine
Date : / /20
Page :

- Determine the average value of titration for both [standard + test soln].
- Now plot the graph shown below for both.



Assay of Vitamin B_{12}

(ii) → **Turbidimetric method** :

→ Use device Photoelectric colorimeter.

- Apparatus, reagent & procedures are same as titrimetric method but in this includes two more test tube which is uninoculated blank tube [not standard + not test soln + no micro organism].

→ Incubate all test tube at 30-37°C for 16-24 hrs.

→ Now, By using 'uninoculated blank tube' adjust the transmittance at 640 mμ to 100% in the photoelectric container.

→ Thoroughly mix the contents of each tube & record the transmittance reading.

Mine
Date : / /20
Page :

→ Now, plot the graph by considering transmittance value against the corresponding level of Standard cyanocobalmin Soln.

→ Draw a smooth curve & calculate the conc. of the test soln of cyanocobalmin.

Q.3 Write various air flow pattern in laminar air flow?

- Laminar Flow Equipments :

→ It is also known as Laminar air flow / Laminar flow Cabinet / Tissue Culture hood / Clean benches.

→ Laminar flow cabinet is an enclosed bench designed to prevent contamination of microorganism / dust at the time of Biochemical testing, performing reaction / test, also use for particles sensitive materials.

→ It consist of a filter pad, a fan [blower] & a HEPA & one laminar air flow bench.

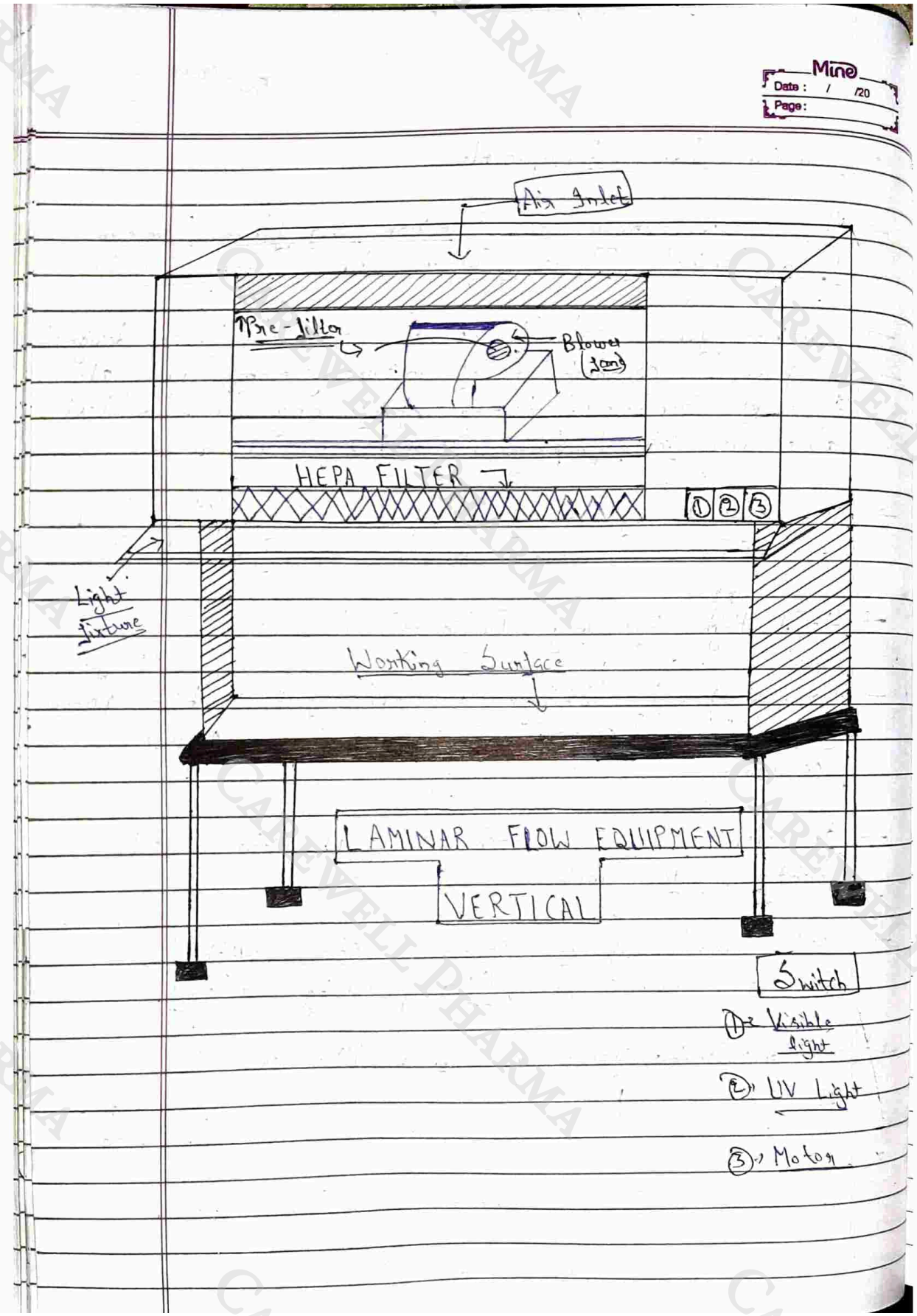
→ HEPA High Efficiency Particulate Air.

→ Air is passed through a HEPA filter which remove all airborne contamination to maintain sterile condition.

→ It also consist switch for UV light, Visible light & for motor.

→ It is made up of Stainless Steal with No gaps or joints [for preventing bacteria].

→ Vertical laminar flow are look like as Pecaano.



Mine
Date : / /20
Page :

- Working :

→ Before starting work on laminar flow firstly on UV light for about 30 min. to sterile the laminar flow.

- Attention → During UV light sterilization, stay away from Laminar flow or UV light, because it cause cancer. [maintain distance from UV light which UV is on].

→ Then wiped the working surface with ~~on~~ ethanol then use it.

→ Then on the motor, the blower suck the air through the filter pad, where dust is trapped.

→ After that the ~~pre~~ prefilter air has to pass the HEPA filter where contaminating microorganism are removed.

→ Sterile air flow into working area.

- Types of Laminar flow Cabinet : → (2)

i). Horizontal laminar air flow.
ii). Vertical laminar air flow.

- Horizontal laminar air flow :

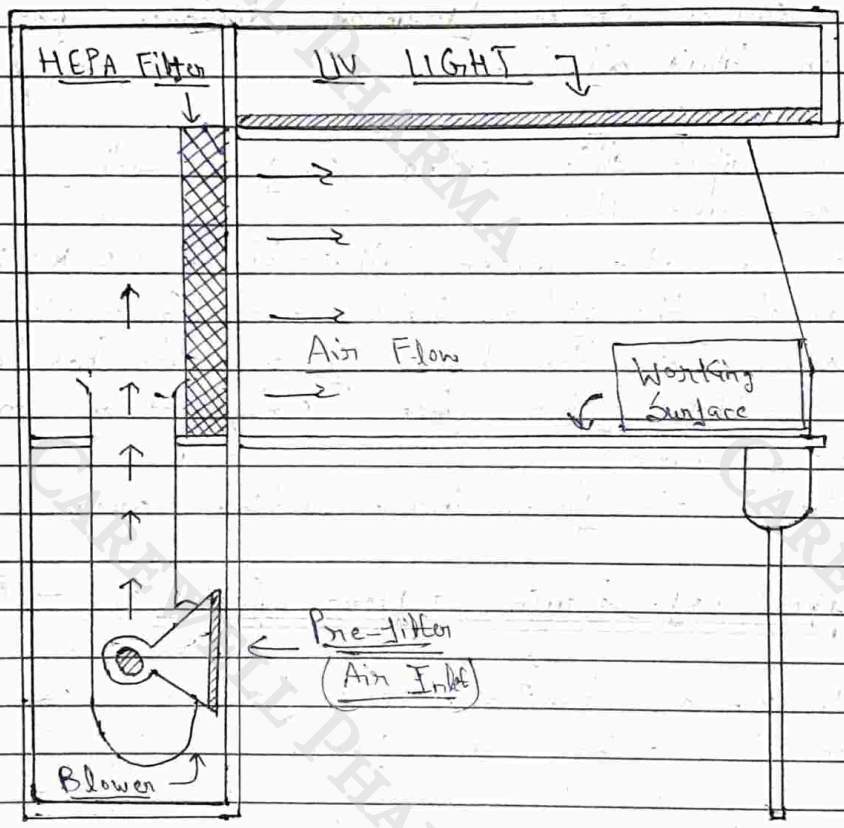
→ In this, air flow from back to front (horizontally).

→ Blower is fitted downward & HEPA filter on front.

- Vertical Laminar air flow :

→ In this, air flow from top to dowand (vertically)

→ Blower (fan) is fitted upward of working area with HEPA filter.



Horizontal Laminar Air Flow

Q-1= Describe in details about animal cell culture [prep^n, maintainance, procedure, applications].

• Animal Cell Culture :-

→ Growth of animal cell in culture, general procedure for cell culture, Primary, established & transformed cell cultures

→ Applications of cell cultures in p'ceutical industry & research.

→ The animal cell cultures is a technique in which cells are obtained from animals, grown & maintained in a suitable medium.

→ The culture produced by the cell or tissue taken from an organism is called as 1° culture.

→ The sequence of culture obtained from the first sub-cultivation of the primary culture is called 'Cell line'.

→ The first attemp to grow animals cells in culture is attributed to Ross Harison in 1907. He was able to cultivate frog embryonic nerve cells using the hanging drop technique.

• Growth of Animals cell in culture :-

→ Design of animal cell culture media is more difficult than that of microorganism & plant cultures.

→ Culture media are used to support the survival as well as growth.

→ Selection of media is dependent on type of cells & main objective of culture.

Culture media classified as:
i) → Natural media
ii) → Artificial / Synthesized media

① → Natural → This media are obtained from natural sources such as plasma clot, biological fluids & tissue extract.

- Blood Plasma → It provide a nutritive substrate & supporting structure for many types of cultures.

- Blood serum → It is also called [Fibrinogen free plasma]
→ It is used in animal tissue culture.
→ It is mixture of plasma proteins, peptides, lipid, carbohydrates, harmones, enzymes & minerals

(ii) → Artificial media → It is prepared by adding organic & inorganic nutrients, vitamines, salts, serum protein, carbohydrates, O_2 & CO_2

- Serum containing media → In which serum is added in 5 to 20% amount.

- Serum free media → These are developed to overcome the limitations of serum.
It has ability to make the medium more selective for a particular cell types.

Q-2. What are preservatives ? Discuss their role in p'ceutical products ?

→ An antimicrobial agent [preservatives] are the chemical substance used to improve shelf life of drug or formulations & inhibit the growth of microbes & reduces the risk of spoilage of p'ceutical products.

→ The main functions of antimicrobial preservatives is to prevent the growth of unwanted micro-organism in p'ceutical prep".

→ The correct approach to prevention has as its foundation in two imp. principles :

- The first of these is that the addition of a preservative to a product must not be done to mask any ~~dit~~ deficiencies in the manufacturing procedures.
- The preservative should be an integral part of the formulation, choosen to afford protection in that particular environment.

→ A single preservative is not suitable for preservation of all p'ceutical formulations; combination of two or more preservatives are used to extend the range & spectrum of preservation.

→ Preservatives are widely employed in p'ceuticals dosage forms such as emulsions, suspension, semi-solids, parental prep". etc.

Mine
Date : / /20
Page :

2. There are some commonly preservatives in different dosage forms:

Preservatives used in pharmaceutical formulations.

Formulations	Preservative
Tablet	Methyl paraben, Phenol, Cresol.
Eye-drops	Benzalkonium chloride, Phenyl mercuric nitrate.
Liquids/ Mixtures	Alcohol, Methyl paraben, chloroform.
Semi-solids	Chlorocresol, Dichlorobenzyl alcohol

Q-3→ What is microbial spoilage ? its types ?

- **Microbical Spoilage** → It is spoilage of any p'ceutical products or drugs due to contamination of microorganism & their products, which further not intended for use

→ P'ceutical products may be considered to be microbiologically spoiled if low level of pathogenic microbes or toxic microbial metabolities are present & detectable physical or chemical changes have occured in the products.

- **Types of microbial spoilage** : (4)

i)→ **Injection induced by contaminated pharmaceutical products** → P'ceutical products may be contaminated by pathogenic microorganisms mainly from raw material or at the time of prep^n, which cause serious infectio

to the patients.

ii) Physio-chemical spoilage → Due to microbial contamination, there are change in physical & chemical properties of drugs.

iii) Observable effect of microbial attack on products →

Microbial spoilage of different dosage form may be detected by Organoleptic tests. There spoilage products may release very unpleasant smelling & tasting metabolities such as 'Sour' 'fishy' etc.

iv) Ingredients susceptible to microbial attack → (3)

a) Therapeutic agents → Many drugs are capable of ~~gross~~ gross degradation by a wide variety of microorganism.

e.g. Aspirin may be converted into salicylic acid & penicillin by [β-lactomase].

b) Preservatives & disinfectants → Most organic & disinfectants are metabolized readily by many bacteria & fungi.

e.g. Pseudomonas species have metabolised 4-hydroxy-benzoate ester preservatives contained in eye drops & caused serious eye infections.

Mine
Date : / /20
Page :

c) → Sweetening, flavouring & colourless agents → Many Sugar & other sweetening agents used in pharmacy are readily substrates for microbial growth.

e.g., Flavouring agents such as peppermint water & chloroform supports help growth of bacteria & yeast.

Q-1 :- Write a short note on preservative efficacy test ?

→ It also known as Preservative efficacy test.

→ This test is applied to the formulated medicines in its final container to determine whether it is protected against microbial spoilage.

→ It is used to check stability of multiple dose such as ~~parenteral~~ parenteral, oral, nasal, topical & opthalamic products made with aqueous base or vehicle.

→ It is used to check the effectiveness of antimicrobial preservatives.

→ These p'ceutical formulation also evaluated at time to time (once evaluated under 6 months).

→ The test & standard apply only to the product in the original, unopened container, in which it is supplied by manufacture.

- Medium used → for the initial cultivation of test microorganism, use Soyabean casein digest agar medium.

Mine
Date : / /20
Page :

- The test microorganism used for preservative efficacy test are...
- Staphylococcus aureus ATCC 6538,
- Pseudomonas aeruginosa ATCC 9027,
- Escherichia coli ATCC 8739,

- Procedure:

→ Inoculate each original product container with one of the Standardised microbial Suspension using a ratio equivalent to 0.1 ml of inoculum suspension to 20ml of product & mix.

→ Final conc. should be $1X10^5$ to $1X10^5$ microbes per ml of products.

→ Determine the no. of viable microorganism by the plate count method & calculated the initial conc. of microbes per ml.

→ Incubate the inoculated containers or tubes at room temp.

→ Determine the viable count by the plate count method at 7, 14 & 28 days subsequent to inoculation.

→ Calculation the percentage of reduction in CFU per ml for each organism at the states test intervals & express the change in terms of percentage of initial conc.

CFU [Colony-Forming-Units]

Interpretation of Result:

- For parenteral, opthalmic, sterile nasal & ~~opthalmic prep~~, otic prep, conc. of viable bacteria is not more than 10% of initial construction of 7 days & not more than 0.1% of initial conc. at 14 days & there is further decreased in count at 28 days.

- For topical prep.

Conc. of viable bacteria is not more than 1% of initial conc. at 14 days & there is further decrease in count at 28 days.

- For Oral → not more than 10% of the initial conc. at 14 days & further decreased.