JRG College of Pharmacy

# JRG COLLEGE OF PHARMACY

# UNIVERSITY SOLVED QUESTION WITH ANSWER

| | | |
|---|---|---|
| **Year** | : | **2019-20** |
| **Subject** | : | **Ph. Microbiology** |
| **Subject Code** | : | **BP303T** |
| **Subject In-Charge** | : | **Mr. Biswajit Biswal** |

**Registration No:** | | | | | | | | | | |

**Total Number of Pages : 01** **B.Pharm**
**BP303T**

**3rd Semester Regular/Back Examination 2019-20**
**PHARMACEUTICAL MICROBIOLOGY**
**BRANCH : B.Pharma**
**Max Marks: 75**
**Time : 3 Hours**
**Q.CODE : HRB671**

**Answer Question No.1 (Part-A) and 02 (Part-B) which are compulsory and any TWO from Part-C.**
**The figures in the right hand margin indicate marks.**

**Part-A**

**Q1** **Only Short Answer Type Questions (Answer All-10)** **(2 x 10)**

a) Differentiate prokaryotes and eukaryotes.
b) Why for streak plate method is carried out?
c) Write the composition of nutrient agar.
d) Draw a suitable design for construction of aseptic area.
e) Define preservatives with few examples.
f) Differentiate disinfectants and antiseptics.
g) What is germ theory of disease?
h) Write four different factors influencing disinfectant action.
i) What do you know about HEPA?
j) Name four different methods for quantitative measurement of bacterial growth.

**Part-B**

**Q2** **Only Focused-Short Answer Type Questions- (Answer Any SEVEN out of NINE)** **(7 x 5)**

**Discuss briefly on the followings :**

a) Phenol coefficient test
b) Growth pattern of bacteria with growth curve
c) Isolation methods for pure cultures
d) Gram's staining
e) Animal cell culture
f) Sterility indicators
g) IMViC tests
h) Replication of virus
i) Classification of fungi

**Part-C**

**Only Long Answer Type Questions (Answer Any TWO out of FOUR)**

**Q3** Describe the structure of bacteria with help of a labeled diagram. **(10)**

**Q4** What is sterilization? Discuss in detail physical methods of sterilization. **(10)**

**Q5** Discuss microbial assay of antibiotics. **(10)**

**Q6** What do you mean by microbial spoilage? Write different sources of microbial contaminants. **(10)**

Pharmaceutical Microbiology
2019-2020

2 marks

1) a- Differentiate prokaryotes and Eukaryotes

Ans

| Characteristics | prokaryotic cell | Eukaryotic cell |
|---|---|---|
| Nucleus | Absent | present |
| Cytoplasm | Absent | present |
| Mitochondria | Absent | present |
| Lysosomes | Absent | present |
| Golgi apparatus | Absent | present |
| Chloroplast (Coin shaped) | Absent | may be present |
| Cytoplasmic membrane | Generally sterols are absent | Sterols are present |
| Cell wall | peptidoglycon present | peptidoglycon Absent |
| eg | Bacteria, Actinomycetes | fungi, protozoa, Algae, plants |

b) Why for streak plate method is carried out.

Ans The streak plate method is carried out to Isolate individual / pure bacterial colonies from a mixed culture.

purpose of the streak plate method:-

i) Isolation of pure culture

-> The primary purpose is to obtain a pure culture, which is a population of bacteria all derived from a single parent cell.

ii) Study of Individual species

-> By Isolating a single species from a mixed sample

microbiologists, can study its specific characteristics such as its morphology, physiology and biochemical properties.

iii) Diagnostic and Research applications

→ Pure cultures are essential for accurate antibiotic sensitivity testing, genetic analysis & other research applications in pathology, ecology & disease study.

c) Write the composition of nutrient agar

Ans Composition of nutrient Agar

1) Peptone

→ 5g/L. An enzymatic digest of animal protein

2) Beef Extract

→ 1-3g/L - water soluble substances providing vitamins, minerals, carbohydrates

3) Sodium chloride

→ 5.g/L maintains the proper salt concentration and osmotic balance within the medium.

4) Agar

15.0g/L. A solidifying agent

5) Yeast extract

→ sometimes Includes as an additional source of vitamins, nitrogen and other growth factors

d) Draw a suitable design for construction of Aseptic Area?

Ans It is classified into 3 areas.

1) General Area
2) Clean area
3) Critical Area

1) Uncontrolled Area / General Area

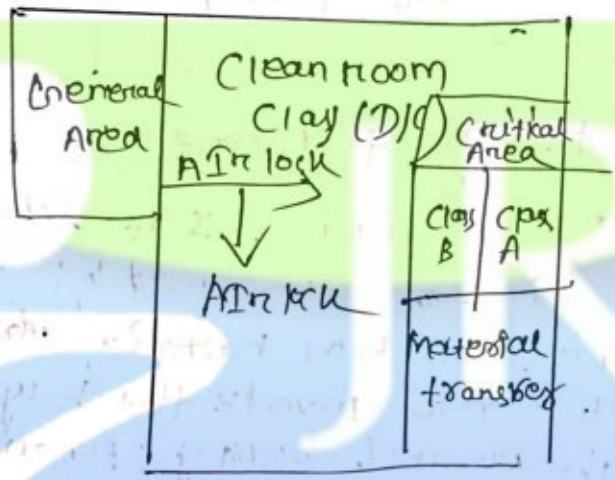
→ Normal working environment outside the cleanroom.
→ Personnel enter here before changing into cleanroom garment.

2) Clean room (Class D/C - Support Areas)

Class-C -> Sterilization of raw material.
Class-D -> Intial preparation of pharmaceutical product
-> used to storage of materials, preparation and Intial processing
-) Acts as a buffer zone between uncontrolled and Critical areas.

3) Critical Area. (Class B & A)

Class-B -> Background environment for aseptic filling.
Class-A -> Laminar air flow work station where critical operations like fillings, stopper, placement and capping are carried out.
-> Airlocks, change room and material transfer areas are Integral to minimize contamination during personnel and material movement.




e) Define preservatives with few examples?

Any the main function of preservatives is to prevent the growth of unwanted microorganism in pharmaceutical formulation.

eg Sodium methyl paraben
Sodium propyl paraben
Phenol
Cresol
Benzyl alcohol
Bromophol
Benzalkonium chloride
Cetyl trimethyl

f) Differentiate Disinfectant and Antiseptics

Ans

| Antiseptics | Disinfectants |
|---|---|
| → Antiseptics are used on living tissues to kill or prevent the growth of micro organisms. | → Disinfectants are used on non-living objects to kill the micro organisms |
| → It does not cause any harm to the living tissues. | → They are harmful to the living tissues & can't be applied to the skin |
| → Antiseptics usually kill all microorganisms | → Disinfectants do not necessarily kill all microorganisms |
| for ex:- 0.2% soln of Phenol. | for ex:- 1% soln of phenol |

g) What is germ theory of disease?

Ans The germ theory of disease is the scientific idea that many diseases are caused by tiny living microorganisms such as bacteria, viruses, fungi or protozoa - that invade the body grow & interfere with normal body functions.

h) Write four different factors influencing disinfectant action?

1) concentration of Disinfectant

→ Higher concentration generally increases effectiveness.

Eg 70% alcohol is more effective than 30% alcohol for disinfection.

2) Contact time
- Longer exposure. Increases microbial kill rate.
- Insufficient contact time may leave microbial viable.

3) Temperature
- Higher temperatures usually enhance the activity of disinfectant.

4) Moisture content
- May disinfectant acts better in moist condition.
- Dry surfaces may reduce the peretration and activity of the disinfectant.

i) What do you know about HEPA?
Ans HEPA stands for High efficiency particulate air. It refers to a type of air filter that meets strict standards for trapping very small airborne particles.

Uses
- It used in Healthcare
- It used in pharmaceutical Industries, labs.
- It also used in transportation.

ii) Name of the different methods of quantitative measurement of bacterial growth.
1) Cell count
2) Direct microscopic count.
3) Quantification via electronic method
4) Plate counting technique
5) Bacteria number estimation based on turbidity.
6) Nitrogen content determination
7) Cell dry / weight determination
8) Bacteria counting using membrane filteration.

5 marks

2a) Phenol Coefficient Test:

Any In this method, a test chemical is rated for its microbicidal property with reference to phenol under Identical conditions.

-> organisms used in this method are Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa

-> Phenol coefficient test includes..

1) Rideal walker Test (RW Test)
2) Chick Martin Test (CM Test)

1) Rideal walker Test

-) The phenol coefficient of Test disinfectant may be calculated by Rideal walker Test that use Rideal walkers broth and Salmonella typhi as the sensitive micro organism.

Procedure

-) Different dilution of the Test disinfectant and Phenol are prepared and 5ml of each dilution is inoculated with 0.5ml of the 24 hour broth culture of the organism.

-) All tubes (disinfectant + organism & phenol) are placed in a 17.5°C water bath

-> Subcultures of each reaction mixture is taken and transferred to 5ml sterile broth after 2.5, 5, 7.5 and 10 min.

-> The broth tubes are Incubated at 37°C for 48 to 72 hours and are examined for the presence or absence of growth.

| Disinfectant | dilution | Time 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 | + | + | + | + | |

(+) = It shows growth
(-) = It shows no growth

-) Now calculate Rideal walker coefficient of the disinfectant.

$$R\cdot W \text{ coefficient} = \frac{\text{Dilution of Test disinfectant killing in 7.5 but not in 5 min}}{\text{Dilution of phenol killing in 7.5 but not in 5 min}}$$

$$R\cdot W = \frac{2000}{100} = 20.$$

-> If the phenol coefficient (R-W coefficient) of given Test disinfectant is

1 -) Same effectiveness as phenol.

Less than 1 -> Less effectiveness than phenol

more than 1 -> more effectiveness than phenol.

* Here is 20, that means the test disinfectant is 20 times, more active than phenol.

2/ Chick martin test

-) This type of Test is done in controlled amount of organic matter in the form of standardization suspension as yeast cell.

-) In this Test all the conditions are like rideal walker Test but the Temperature is 20°C and the exposure time is 30 mins.

| Disinfectant | Dilution | Time Interval for subculture | | | |
|---|---|---|---|---|---|
| Test disinfectant | 1:1000 | + | - | - | - |
| | 1:2000 | + | + | - | - |
| | 1:3000 | + | + | + | - |
| | 1:40000 | + | + | + | + |
| Phenol | 1:80 | + | - | - | - |
| | 1:100 | + | + | - | - |
| | 1:120 | + | + | + | - |
| | 1:140 | + | + | + | + |

(+) -> It shows growth
(-) -> It shows no growth.

Chick martin coefficient = Dilution test disinfecting killing in 7.5 but not in 5 min / Dilutions phenols killing 7.5 but not in 5 min

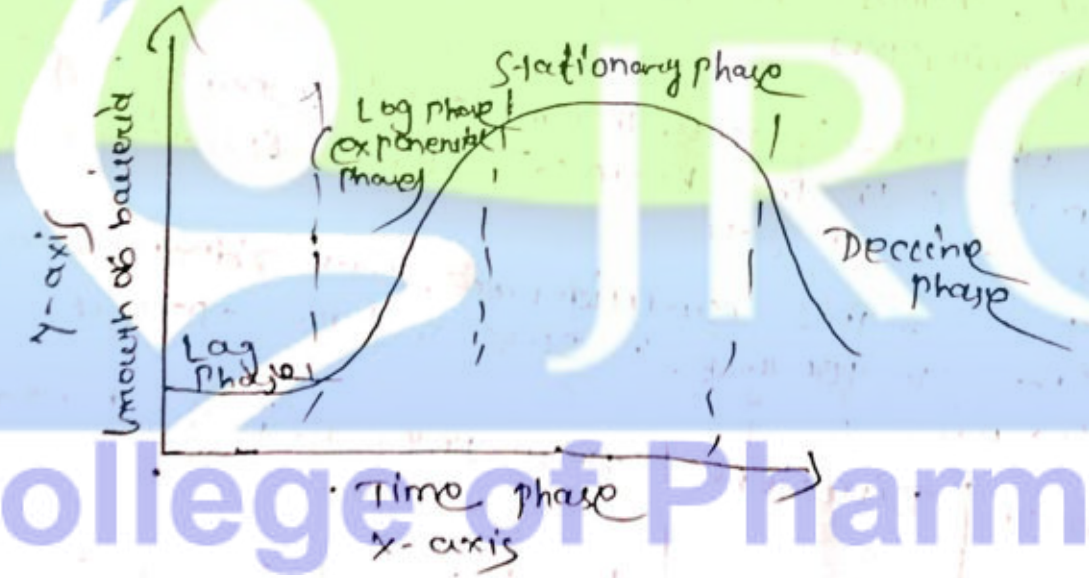
$$C.m = \frac{2000}{100} = 20$$

b) Growth pattern of bacteria with growth curve:

Ans Bacteria growth curve

-) when a graph plot between the growth of bacteria and time phase is called growth curve.
-> The resulting curve has four distinct phases.

1) Lag phase
2) log phase
3) Stationary phase
4) Decline phase




1) Lag phase

-> In this phase, there are no growth of bacteria because culture media is just prepared, so up to 2-3 hour there are no growth of bacteria only nutrition medium present.

2) Log phase

-) In this phase, their all maximum growth of bacteria because after the germination of bacteria, they Reproduce in fast rate and bacterial increases and their are nutrition are more than the bacteria, so bacteria grow maximum.

-) Also called as exponential phase.

3) Stationary Phase

-) In this phase, Growth of bacteria stop, and it may be stationary, because number of birth of bacteria is equal to the number of death of bacteria

-) It is just happend due to lack of nutrition.

4) Decline phase

-) Also known as death phase, in this phase, Bacteria start dying, because no. of bacteria is ~~equal to the~~ no. more than nutrition medium.

-) so due to lack of nutrition, bacteria start dying.

c) Isolation methods of pure culture

Ans 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 separating of a particular microorganism from the mixed culture.

-) It is used to study about specific one species in their own pure culture for growth of any one species

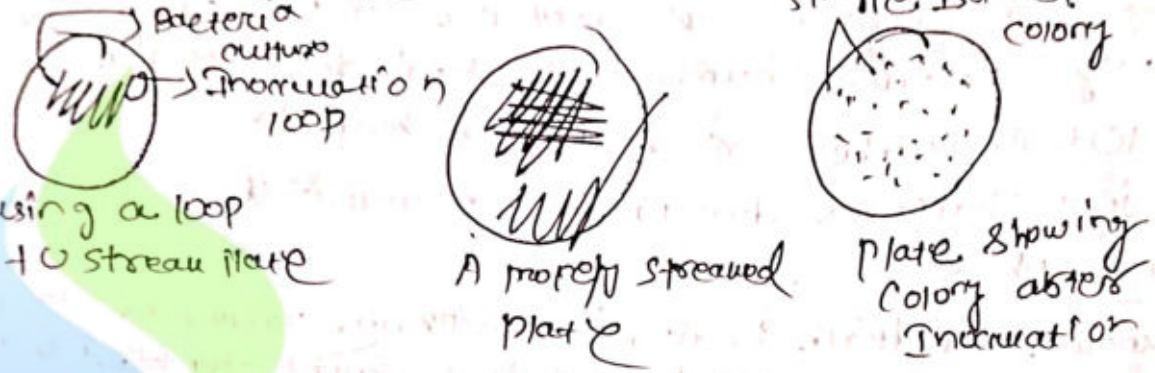
-) The most commonly used methods for pure culture.

1) Streak plate Technique
2) Pour plate Technique.
3) Spread-plate technique
4) Serial dilute Technique.

1) Streak plate Technique

→ In this method, the bacteria is isolated from mixed culture media by applying streak on the surface of culture media with the help of Inoculation loop.

→ Firstly Inoculation loop is heated for sterilization and then it streak on the surface of mixed then it streak on new sterile culture media




using a loop to streak plate

A properly streaked plate

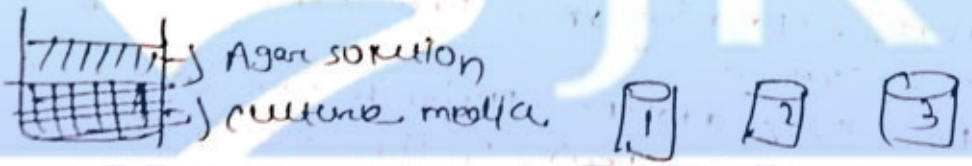
Plate showing colony after Incubation

[isolated colony of bacteria]

2) Pour plate Method

→ In this method, dilute Agar solution (Agar ml) dilute) is mixed in the mixed culture media and dilute the mixed culture media.

→ Now these dilute culture media after proper mixing, it transferred (pour) into petriplate




dilute solution.

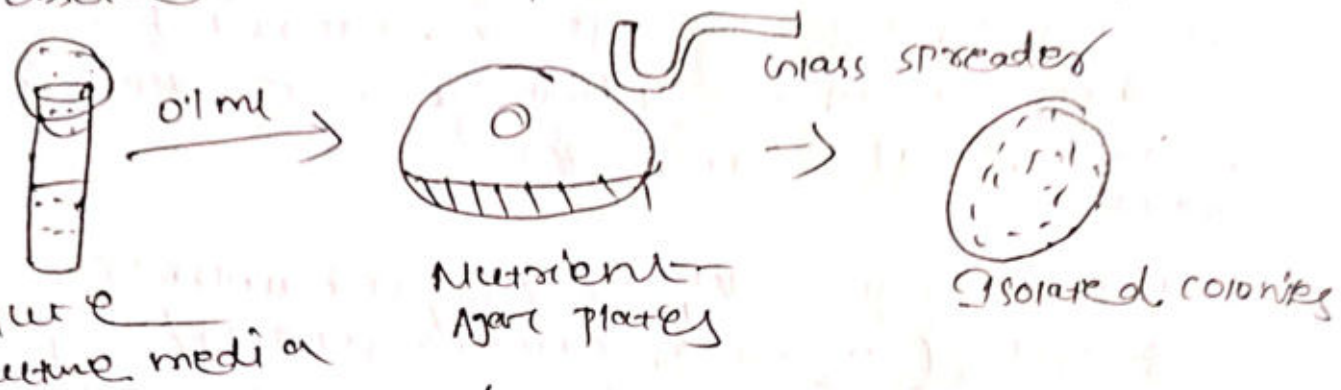
→ the colonies of similar shape, size and colour will be visible.

3) Spread plate technique

→ In this method a mixed culture media is diluted with some sterile water or a saline (salt water) solution.

→ Now take 0.1 ml of culture media drop and placed on the surface of agar plate then spread it with the help of glass spreader ok all over the surface

→ Then allow bacteria to grow and Isolated colonies all observed after 24 hours and counted




4) Serial dilution Method

→ In this method, take five Test tube with filled 9ml of sterile distilled water.

→ Now take 1 ml solution from original suspension and dilute it in 1st test tube, then take one ml from 1st and diluted it in 2nd, then take from 2nd, Repeat this process until all Test tube should be diluted.

→ After diluted all tubes, take 1 ml of each Test-tube & pour into nutrient agar plates.

→ As number of Test tubes increases, the concentration of bacteria is decrease & the dilution is increase.

→ This method is used for actinomycetes bacteria).


d) Gram Staining:

→ It developed by Han's Christian gram in 1884

→ It is widely used differential staining technique which is used for identification & classification of bacteria.

→ In this staining, we use two or more dye at a time.

→ On the basis of structure, bacteria is classified into two major groups i.e. Gram (+ve) & Gram (-ve) bacteria.

Principle

-) It is based on the principle that colour, dye, stains retain only on the gram positive bacteria due to thick layers help in the identification of bacteria as the Gram (+ve) & Gram (-ve)

Procedure

-) Firstly take glass side, cover slip, Inoculation loop culture media (in which bacteria present) & sterile
-) Now wash all these with ethanol solution, then allow for drying, then sterile through flaming.
-) It involves 7 steps

1) Preparation of smear

-) Firstly take Inoculation loop, streak to culture media, then spread bacteria sample onto a slide

2) Fixation

-) Now, slide is allow to air dry then slide is rapidly passed through a flame for three or four time for heat fixation

3) Primary staining

-) Firstly add crystal violet dye & allow 1-2 min react with bacteria & allowing for drying.

4) Decolorization

-) Now wash the slide with ethanol & acetone (to use 1 min) [Gram (-)ve bacteria have thick peptidoglycan layers so on washing with ethanol or acetone, crystal violet dye remains all stays but Gram (+)ve bacteria have thin peptidoglycan layers so dye washed away with ethanol]

5) Counter Staining

-) Now again add Safranine dye & allow 1-2 min to react with bacteria & allow for drying.

6) Washing

-) wash with water to remove extra staining & allow for drying

7| microscopic observation

→ Now observe it on microscope

observation

1) Gram - positive Bacteria

→ purple / blue colour / because crystal violet detached on washing, so safranine not attached

2) Gram - negative bacteria

→ Red or pink colour, because crystal violet detached on washing with alcohol, so on counter staining safranine attached on it & give red or pink colour.

Applications

→ Identification of bacteria morphology

→ classification of bacteria into Gram (+ve) & Gram (-ve)

→ Detection of bacteria contamination, Research & development

e) Animal cell culture

Cell culture

→ cell culture refers to the removal of cells from animal or plant and their subsequent growth in a favourable artificial environment.

→ This cell may be removed from the tissue directly and disaggregated by Enzymatic or mechanically means before culture or they may be derived from a cell line or cell strain, that has already been established.

→ The cell culture technique was suggested & discovered by Prof Wenville Harrison in 1907 who successfully grown frog nerve cells in clotting lymph medium by using hanging drop method.

Classification

→ cell culture is further classified into 3 types, on the basis of origin, Chromosomal Characters and the number of generation.

1) Primary cell culture

2) Diploid cell culture

3) Continuous cell culture.

1) Primary cell culture

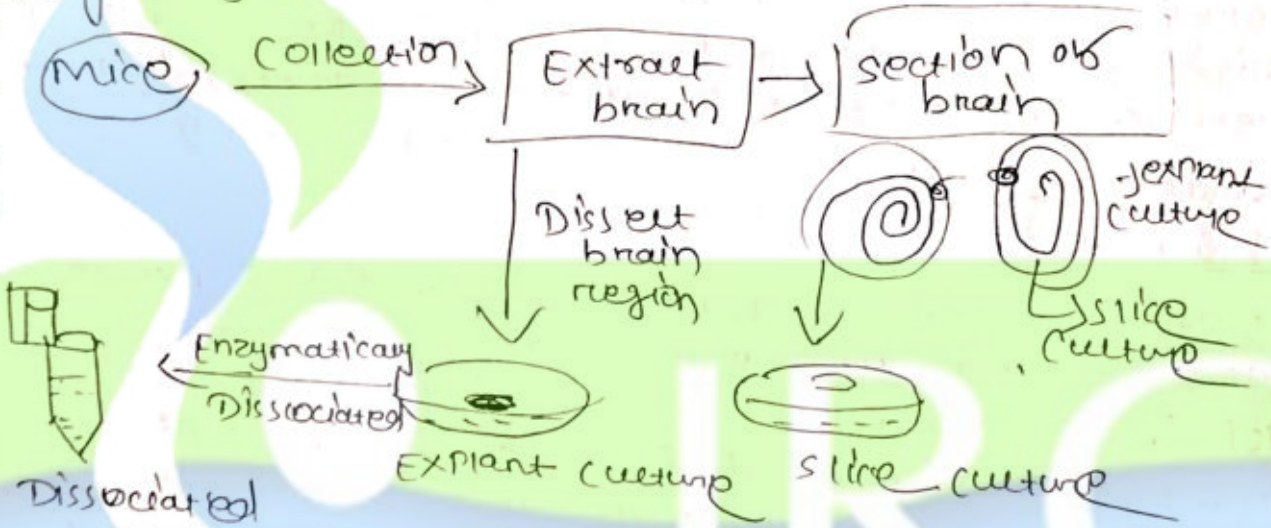
-) These are the normal cells freshly taken from the body & the culture.

-) There capable of only limited growth in culture

-) They can't be maintain in serial culture

eg monkey's kidney, Human embryo kidney, chick embryo cell culture.

-) Primary culture are directly derived from normal animal tissue & cultures either as explant culture or dissociation into a single cell suspension by enzymatic digestion




2) Diploid cell culture

-) Diploid cell strains are of single type that retain the original diploid chromosomal number & karyotype during serial sub cultivation for a limited number of times.

-) After above serial 15 passage, they undergo senescence.

eg the diploid strain develop from human fibroblast.

3) Continuous cell line culture

-) These are the cells of single type, usually derived from cancer cell. they are capable of continuous serial cultivation indefinitely

eg carcinoma of cervix.

-) cell culture is used for Isolation virus and their cultivation of vaccine production.
-) Tumour cell like are often variety from actual clinical tumors but transformation may also be induced by viral oncogenes or by chemical treatment

General procedure for cell culture

-) Incubation temperature should be 36°C
-) The pH for growth should be 7.2-7.4.
-) The label of glucose & L-glutamine Influence the growth of the cell.
-) The range of Inorganic ions & vitamins are essential for cell survival.

f) Sterility Indicators:

Any These Indicators desired the efficiency of an Sterilization process.

Types

-) There are 3 types of Sterility Indicators used in evaluation of Sterilization method

1) Physical Indicator
2) Chemical Indicator
3) Biological Indicator

1) Physical Indicator

-) The display on the sterilizer or a recording that print out the parameter like Temperature & pressure associated with each sterilization cycle
-) The correct reading of the Physical Indicator do not ensure the sterilization process.
-) But If Incorrect Indication problem in Sterilization Cycle & the load may not be sterilized

ex

Methods

1) Dry heat sterilization
2) moist heat sterilization
3) Vapour Sterilization
4) Radiation Sterilization
5) Filtration Sterilization.

physical Indicator
Temp. Record chart,
Temp. Record Chart.
Temp. Record Chart
Radiation measure chart.
Bubble point pressure/
Vapour pressure

2) Chemical Indicator

-) These are the chemicals used in the process of sterilization in order to indicate the process of sterilization is going as per the requirement or it may be indicates the products are sterile.

eg

Depending upon the sterilization process the chemical indicators are selected.

i) The chemical indicator that are used for dry heat & moist heat sterilization, may change it's colour only when satisfactory condition for sterilization prevailed.

ii) This confirm the successful completion of sterilization cycle.

iii) In case of gaseous sterilization Royach sacket (Indicator paper) is used, which is stained with reactive chemical, It's role is to show a colour change / indicate the prevailing condition.

3) Biological Indicator

-> In case of biological indicator we use standardized bacterial spores used in the form of suspension in water or culture media. or it's dried form on paper or plastic & kept in the sterilization.

->The Bacterial spores are selected as per the process used for sterilization such as spores of Bacillus subtilis is used as biological indicator in dry heat sterilization to determine D value.

-> Bacillus thermophilus used in case of moist heat sterilization.

Ex

| Methods | principle | Microbes | Parameter |
|---|---|---|---|
| 1) Dry heat sterilization | Temp. sensitive microbes | Bacillus subtilis | D-value |
| 2) moist heat sterilization | Temp. sensitive microbes | Bacillus thermophilus | D-value |
| 3) Gaseous sterilization | Temp. sensitive microbes | Bacillus stereo thermophilus | D-value |
| 4) Radiation sterilization | Radioactive sensitive microbes | Bacillus pumilus | D-value |
| 5) filteration sterilization | Retaintion of microbes | Pycno clavella diminuta | Size of bacteria |

g) Imvic Test ?

-> It is a series of four biochemical test used to Identify & differentiate enterobacteriacea family bacteria.

-> It also known as Biochemical Test

-) There are some kind of bacteria, which have same size, shape & we can't identified through staining on microscope. So identification of that type of bacteria are formed by chemicals.

-) Imvic stands for,

- -> Indole Test (I)
- -) Methylene Red Test (M)
- -) Voges - Proskauer Test (VI)
- -) Citrate test (C)

1) Indole test

-> Detects the ability of bacteria to produce Indole from the amino acid tryptophan

-> A positive result is indicated by the formation of Red or pink ring when reagent (reagent) is added after Incubation.

Tryptophan → Indole → Pyruvic acid + ammonia ($NH_3$)

Procedure

→ Inoculate tryptophan broth (peptone water) with test microorganism (bacteria) then Incubate 37° for 24-28 hours.

→ Then add Kovac's reagent (10-15 drops) mix gently.

→ Red colour ring formation indicates the production of Indole.

→ If, Indole produced (red ring) → Test +ve indicates that test bacteria is E.coli Negative no-colour change.

2) Methylene blue Test

→ It is based on the principle that during fermentation of glucose, acid produced and red colour indicates positivity of Test.

→ Inoculated the Test microorganism (bacteria) in glucose phosphate broth then Incubate at 37°C for 24-28 hour.

→ Then add methyl red indicates (5-7 drops) mix gently.

→ Red colour indicates acid production & positivity of Test.

Test positive → E.coli bacteria

Test Negative → E. aerogenes bacteria (No colour change)

3) Voges-Proskauer Test

→ It is based on the principle that acetoin (acetyl methyl carbinol) produced from glucose fermentation & give pink colour (indicates Test positivity).

→ Inoculate Test bacteria into glucose phosphate broth & Incubate at 37°C for 24-48 hours

→ Add 1ml potassium hydroxide (KOH) & 3 ml of 5% solution of α-naphthol in absolute alcohol i.e Barritis Reagent A & B.

-> if pink or Red colour (acetoin produced) Test (+)ve -> E. aerogens bacteria
no colour change -> Test negative -> E.coli bacteria

4) Citrate Test

-> It is based on the principle that, In this we check the ability of bacteria to utilize citrate as a carbon source for growth.
-> Inoculate Test bacteria Into Kosers citrate medium (contain citrate). Now bacteria uses citrate as carbon source & growth himself and this is Indicates by the production of turbidity in the medium,
-> if turbidity produced -> Test (+)ve -> E. aerogen
-> if no change -> Test (-)ve -> E.coli bacteria

b) Replication of virus.

-> when virus Insert on host cell, a host cell is found to rapidly produce thousand of Identical copies of original virus.
-> It takes place through various Stages.

1) Attachment
Desination
The virus bind to specific receptor molecules on the surface of the host cell.
Mechanism
-> viral surface proteins (spikes) capsid proteins of glyco-protein) recognize & attach to complementary receptors on the host cell membrane
Importance
-> Determines host range & tissue tropism (which species & cells the virus can Infect).
eg
-> HIV binds to CD4 & CCR5 / CXCR4 Receptors on helper T cell.

2) Entry (Penetration)
Desination
The viral particle or It's genetic material enters the host cell.

Mechanism

Endocytosis

→ The host cell engulfs the virus in vesicle.

Eg, Influenza virus.

· Membrane fusion

→ viral envelope fuses with host membrane, releasing contents into cytoplasm,

Eg HIV.

· Direct penetration

→ Genome is injected into cell.

Eg Bacteriophages

3) Uncoating

→ Definition

→ Removal of the viral capsid to release the nucleic acid.

Mechanism

→ Lysosomal enzymes / host cell enzymes or viral enzymes breakdown the capsid.

Result

→ viral genome becomes accessible for replication & transcription.

4) Replication

Definition

→ production of new viral genomes using host or viral enzymes.

Differences

i) DNA viruses

→ often replicate in the nucleus using host DNA dependent DNA polymerase.

Eg adenovirus.

ii) RNA virus

→ usually replicate in cytoplasm using viral-RNA dependent RNA polymerase.

iii) Retro virus

→ Reverse transcribe RNA into DNA using Reverse transcriptase, then Integrate into host genome

Eg HIV

5) Transcription and Translation (protein synthesis)

Definition

- viral genes are transcribed into m-RNA & translated into proteins.

Products

Structural proteins (capsid, enveloped proteins)

Non structural proteins (enzymes, Regulatory proteins)

6) Assembly (Maturation)

Definition

- Newly synthesized genomes and proteins are assembled into new viral particles (virions)

Location

Cytoplasm (most RNA viruses)

Nucleus (most DNA viruses)

7) Release

Mechanism

1) Lysis

Host cell rupture, releasing virions (non-enveloped virus some bacteriophages)

Budding

- Viruses acquire an envelope from the host cell membrane as they exist (enveloped viruses like HIV, Influenza)

Effect

- often leads to host cell death or persistant infection

i) Classification of fungi?

These are classified into two ways.

i) Morphological classification

ii) Taxonomical classification.

1) Morphological classification

- Based on the morphology fungi is classified into 4 types.

1) yeast
2) yeast like fungi
3) mould
4) Dimorphic fungi

a) Yeast
-) These are unicellular fungi in which buds remains attach.
-) These are oval or spherical shape.
-) They have granular cytoplasm with a prominent Nucleus
-) These forms circular, smooth & creamy colonies on culture media.
-) These growth at a temperature of 30-37°C.
eg i) Cryptococcus neoformans
ii) Saccharomyces cerevisiae.

b) Yeast like fungi
-) In some yeast, the bud remains attached to mother cell & elongated, followed by repeated budding forming chains of elongated cells known as pseudo hyphae
-) Forms moist, creamy colonies on media.
eg Candida albicans.

c) Moulds
-) Fungi which form mycelia or filaments are called mould or filamentous fungi.
-) The body of a mould consist of filaments of cells joined together & these are called hyphae & also contain spores.
-) Each hyphae is about 2-10 micrometer wide
-) It is multinucleated in nature.

d) Dimorphic fungi
-) Dimorphic fungi are fungi that can exist in two different morphological forms depending on environmental conditions especially Temperature

| Condition | Morphological form | Description |
|---|---|---|
| Room temperature (25°C) | mould (mycelial form) | multicellular filamentous hyphae |
| Body Temperature | yeast form | unicellular oval shaped. |

2/ Taxonomical classification

-) Taxonomy means on the basis of naming, arrangement characters / Reproductive / structural / life cycle these are classified into 6 major phylum.

a). Phylum Chytridiomycota (chytrids)

-) mostly Aquatic fungi
-) these are unicellular or multicellular
-) produce sexual & Asexual spores with flagella
   eg Allomyces, Synchtrium.

b) Phylum zygomycota (zygote fungi)

-) mostly Terrestial.
-) produce zygospores through sexual reproduction.
-) Non- septate hyphae.
   eg Rhizopus, mucor

c) Phylum Glomeromycota

-) forms symbiotic relationship with roots of plants
-) Helps plant in nutrient uptake, especially Phosphorous.
   eg Glomus.

d) Phylum Ascomycotai (sac fungi)

-) Largest phylum of fungi.
-) produce sexual spores in sac.
-) Asexual Reproduction.
-) septate hyphae
   eg Saccharomyces, penicillium, Aspergillus.

e) Phylum: Basidiomycota (Club fungi)

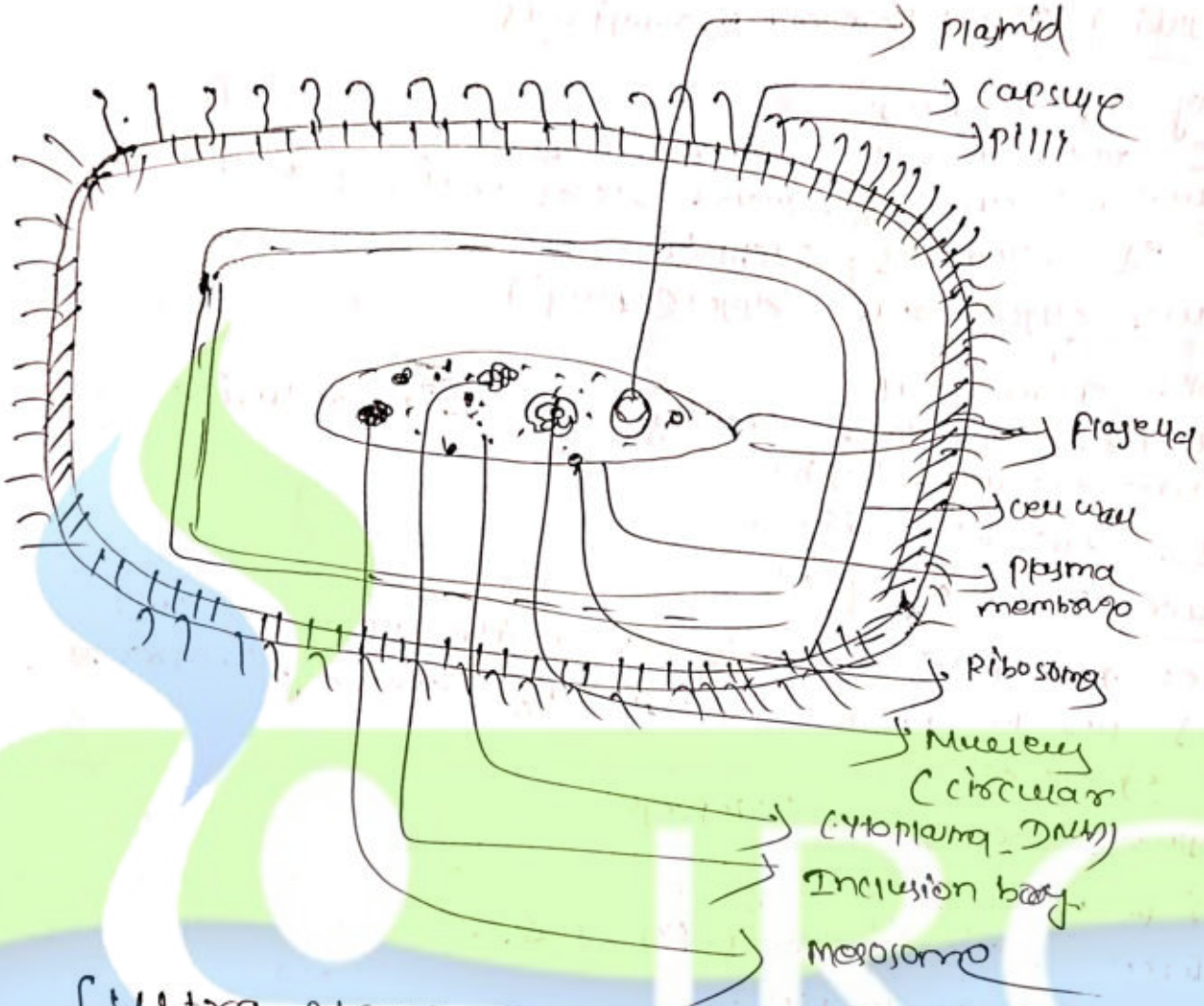
-) produce sexual spores caved Basidiospores (club shaped)
-) Asexual Reproduction in rare or absent.
-) septate hyphae.
   eg Agaricus, puccinia

f) Phylum Deuteromycota (fungi Imperfecti)

-) Asexual Reproduction only.
-) Include many pathogenic fungi.
   eg candida, trichophyton.

5 marks

3) Describe the structure of bacteria with help of a labelled diagram.




[Ultra structure of bacteria]

→ The key feature of ultra structure of Bacteria include a cell membrane, cytoplasm a rigid cell wall and a nucleoid containing circular DNA.

→ Bacteria can also have external structure like flagella for movement, pili or fimbrae for adhesion & a capsule for protection.

Internal structure

1) Cytoplasm

→ The gel like substance within the cell of plasma membrane which carries all cellular organelles and these activities occur.

2) Nucleoid
-) The region containing bacterial chromosome, which is a single circular DNA.

3) Ribosomes
-) Sites of protein synthesis.

4) Inclusion Bodies
-) storage granules for nutrients or other substances.

5) Cell wall
-) A rigid structure composed of peptidoglycan, which protects the cell from osmotic damage and maintain its shape.

External structure

1) Flagella
-) Long whip like appendage used for motility or movement.

2) Pili (Fimbrae)
-) short hair like appendages used for attachment to surface or other cells.

3) Capsules
-> A polysaccharide layer outside the cell wall, providing protection and contributing to virulence (the degree to which the pathogen can cause damage or disease in a host).

Variation
Endospore
-) this is dormant, highly resistant structures that some bacteria form under difficult condition.

Mesosomes
-) Invagination (a pocket form inward) of cell membrane. Involved in DNA replication and cell division.

Q) What is sterilization? Discuss in detail physical methods of sterilization.

Sterilization

→ Sterilization is obtained by microorganism such a way to antimicrobial agent for sufficient time at optimum condition to kill or Inhibit the microorganism.

Types

Sterilization

- Physical
  1) Heating
  2) Filteration
  3) Radiation
- Chemical
  1) Liquid substance
  2) Gaseous substance

1) Heat Sterilization

→ Heat sterilization is most effective & widely used method of sterilization where the bactericidal activity results through the destruction of enzymes & other essential cell constituents.

→ The effect of heat sterilization occur more rapidly in a fully hydrated state, as it requires a lower heat input with low temperature & less time at humidity condition where the denaturation & hydrolysis at predominantly occur rather than in the dry state where oxidative changes takes place.

→ This method of sterilization is applicable for thermostable product.

Types

A) Dry heat sterilization
B) moist heat sterilization.

A) Moist heat sterilization

→ Moist heat sterilization is one of the most important & effective sterilization where the steam under pressure acts as a bactericidal agent..

-) moist heat sterilization usually involves the use of steam at temperature 121°C at 15 lbs pressure for 15 mins.

) High pressure increases the boiling point of water & thus help's achieve a higher temperature for sterilization.

-) The high temperature short time cycles not only results in microbial degradation & also higher level of sterility Assurance.

) The most commonly use standard temperature time cycle for clinical specimen (surgical dressing) 134°C for 3 mins with 15 lbs & for bottle fluid 121°C at 15 lbs pressure for 15 mins)

-) An autoclave is a device that works on the principle of moist heat sterilization to the generation of steam under pressure.

) In this method the microorganisms are killed by coagulating their proteins & this method is much more greater than dry heat sterilization.

Applications

) In the pharmaceutical & medical sector / processing It is used. In the sterilization of dressing / surgical & diagnostic equipment / container / aq. Injection, ophthalmic preparation.

) In agriculture sector most of the Irrigation fluid other contaminated items.

pasteurization

) pasteurization is a sterilization technique in which all non spore forming microbes are killed in milk by subjecting to a temperature of 63°C for 30 min (Holder method) or 73°C for 20 sec (Flash method)

) It suggested by Louis Posture.

→ In pasteurization however not all the pathogenic microorganisms are killed the major principle of this process is the Reduction in number of viable microbes so that they can no longer cause disease.

Applications

→ In diary (milk) Industry used for sterilization of milk.

→ In pharmaceutical sector used for sterilization of vaccines, serum & toxoids

Tyndalization

→ It is a method of sterilization that is used for sterilization of media with sugars gelatin at 100°C for 30 mins on three successive days.

→ so as to preserve sugar which might get decomposed at higher temperature.

sugar $\xrightarrow{\text{decomposed}}$ Alcohol

B) Dry heat Sterilization

→ Dry heat sterilization is a process in which the removal of microorganisms occurs by applying moisture free heat which is appropriate for moist sensitive substances.

→ The dry heat sterilization is the process that is based on principle of conduction. that is heat is absorbed by the outer surface of the sample & then passed onward to the next layer.

→ ultimately the entire sample reaches the proper temperature needed to achieve the sterilization.

→ The dry heat destroy the microorganism by causing denaturation of proteins & also lyses the proteins in many organism causes drying of cells & oxidative free radical damage.

The temperature range for dry heat sterilization

→ (160 - 180°) 170°C for 2 hours,

→ 150°C for 150 min.

Incineration

-> It is the process of sterilization along with a significant reduction in the volume of the waste

-> It is usually conducted during the final disposal of hospital & other wastage.

-> This process is conducted in a device called Incinerator

Applications

-> It is used for sterilization of material which are difficult to sterilize moist heat sterilization.

-> These substances like powder, oil & other related substances sterilized by dry heat sterilization.

3) filteration

-> It is also one type of sterilization.

-> It is a process of sterilization used for sterilization to remove microorganism without destroying them

-> It is capable of preventing the passage of both viable & non-viable particulates & can be used for both clarification & sterilization of liquid & gases.

-> The primary mechanism involved in filteration are 1) sieving
2) adsorption
3) Trapping.

-> filteration uses membrane filtered that have tiny pores that let/allow the liquid pass but prevent the bigger particles such as bacterial from passing through the filters

Applications

-> The principle applications of sterilizing grade filter system. It sensitive injection of ophthalmic preparation, biological product (vaccine, Toxoid, serum) & other cases for supplied aseptic area.

-> In Industry sector, It has important application where these are the part of autoclave / freezedryer, fermentors / centrifuge.

4) Radiation

→ Radiation is the process of sterilization in which the exposing of surfaces objects to different kinds of Radiation for sterilization.

→ mainly electromagnetic Radiation is used for sterilization.

→ The major target for these Radiation is considered to be microbial DNA where the damage occur as a result of Ionization & free radical production or excitation.

U.V Radiation

→ ultra violet Radiation includes light rays from on 150 - 3900A° from which 2600A° as the bactericidal activity.

→ upon exposure, these waves are absorbed by many materials particularly Nucleic acids, they wave as a result cause the formation of pyrimidine dimer. which bring error in DNA replication & cause the death of microbes by mutation.

Non-Ionising Radiation

→ Non-Ionising Radiation Includes Infra-red Radiation & U.V radiation comes under this type of Radiation.

→ Infra red Radiation is used for mass sterilization of syring & catheters.

5) Discuss Microbial Assay of Antibiotics.

→ The microbiological assay of antibiotics is a bio assay method that estimates the potency or concentration of an antibiotics Sample by measuring it's Inhibitory effect on the growth of suitable micro organism under standardized condition.

→ unlike chemical methods, microbiological assay measure biological activity which is directly related to therapeutic effect.

→ There are two methods which are generally employed for the Assay.

1) Cylinder plate or cup plate method
2) Turbimetric or Tube Assay method.

## Preparation for Antimicrobial Assay

→ preparation of culture Media → mueller Hinton Agar
→ selection of test microorganism - Bacillus pumilus, Staphylococcus epidermidis.
→ preparation of test & standard solutions of Antibiotic

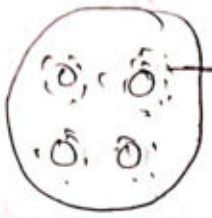
### 1) Cylinder plate or cup plate Method

→ This method depends upon diffusion of an antibiotic from a vertical cavity or cylinder through a solidified agar layer in petriplates.
→ The growth of test microorganism is inhibited entirely in a circular area or zone around the cavity containing a solution of Antibiotics.
→ Three layers plates are prepared for this method

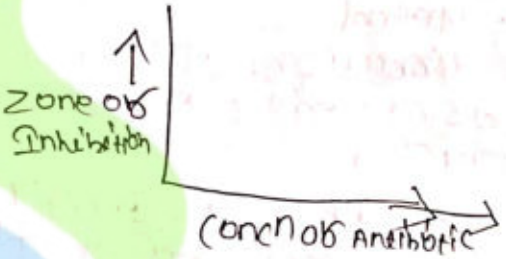

→ Now holes (cavities) are made in the Agar media by using cork bore and antibiotic solutions are added in those holes with the help of micropipettes.


→ solutions of known concentration of standard and test Antibiotics are prepared in appropriate manner (Aliquot
→ Then these solutions are added into sterile cavities in the Agar medium separately (separate for test & Standard)
→ These plates are left standing for 1 to 2 hours at room temperature.
→ All plates are then Incubated for about 18-24 hours.

→ The diameter / Areas of circular Inhibition zones produced by standard & Test Antibiotics solution are accurately measured.




→ Then a standard curve representing concentration of Antibiotic in the x-axis and diameter of zone of Inhibition in y-axis is plotted.




2) Turbidimetric or Tube Assay method

→ Here a liquid medium is inoculated with the test microorganism and different concentration of test & standard antibiotics are added to it.

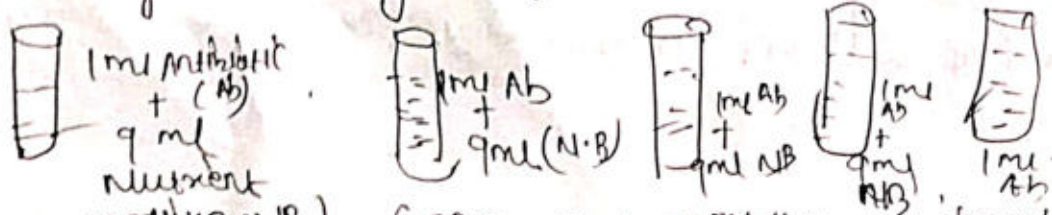
→ Then the growth of the microorganism is Inhibited in proportion to Antibiotic is measured ~~spectro~~ spectrophotometrically in the form of optical density.

→ first a nutrient broth medium is inoculated with a standardized microbial suspension.

→ Then 5 different concentration of Standard Antibiotic solution & test antibiotic solutions are made

→ Now one ml of each concentration of test antibiotic and standard Antibiotic solutions are added into different Test tube.

→ To each tube 9ml of Nutrient medium (containing micro-organism) is added.



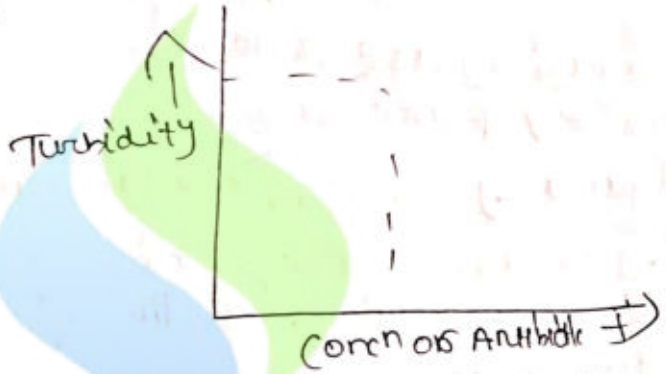

(Separate for Test & Standard)

-) All the tubes are placed in the Incubator at 37° for 3-4 hour or 15-24 hour.

-) Now after the Incubation measure the growth of bacteria into the form of turbidity Spectrophotometrically

-) Then compare the turbidity of Test Antibiotic In comparison to standard.

-) Now make a standard curve relating concentration of Antibiotic at the x-axis & turbidity observed on y-axis.




6/ What do you mean by microbial spoilage? write different sources of microbial contaminants?

Microbiological spoilage

-) Pharmaceutical products are considered microbiologically spoiled when even low levels of pathogen & microbes or toxic microbial metabolites are present and physical or chemical changes have been noted in the product.

-) These microbes not only spoil the pharmaceutically but also food & other consumables. These spoilage of pharmaceutical product can results in serious health risk.

Source & Types of contaminants

-) contaminants can get entry into the production process from several process, such as atmosphere, water raw material, process operators, equipments, packaging, buildings..

1) Atmosphere

-) The number of microorganism in the atmosphere depends on the activity in the environment & the amount of dust which is present.

-) micro organisms are carried into the atmosphere & suspended on particles of dust, then droplets of moisture, sputum from coughing & sneezing.

Eg microorganism like bacteria & fungus such as bacillus species, streptococcus species and penicillium.

2) Water

-) water is one of the main constituents of many products & It is used for processing, washing & cooling purpose.

→ Different types of micro organisms are present in fresh water & some microbes from the water sewage (waste drainage) which contaminate the water of submerged, ocean water & rivers. those are main supplies of Pharmaceutical Industries.

Eg clostridium species, E.coli.

3) Raw Material

-) Raw material are rich source of micro organisms synthetic raw materials are usually free from microbes, but contamination may occur.

-) Raw material mainly contain bacteria & fungi for example, bacillus & Salmonella species but the product from animal sources such as gelatin, pancreas & other tissues may be contaminated with some of the pathogenic micro organisms -

4) Process operators

-) micro organism may be transport to Pharmaceutical formulation from process operators as a results contamination occurs due to poor & Insufficient. Personal hygiene.

Eg staphylococcus bacillus.

5) Equipments

→ The equipments consist of minimum junction valves pump to allow cleaning by circulating of detergents & other chemical having antimicrobial property & disinfectant property.