JRG College of Pharmacy

JRG COLLEGE OF PHARMACY

UNIVERSITY SOLVED QUESTION WITH ANSWER

Year	:	**2022-23**
Subject	:	**Ph. Microbiology**
Subject Code	:	**BP303T**
Subject In-Charge	:	**Mr. Biswajit Biswal**

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

Total Number of Pages : 02

B. Pharm
BP303T

3rd Semester Regular/Back Examination: 2022-23
SUBJECT : PHARMACEUTICAL MICROBIOLOGY
BRANCH: B. Pharma
Time : 3 Hour
Max Marks : 75
Q. Code : L699

Answer Question No.1 (Part-1) which is compulsory, any seven from Part-II and any two from Part-III.

The figures in the right hand margin indicate marks.

Part-I

Q1 **Answer the following questions :** **(2 x 10)**

a) Discuss about the nutritional requirements for bacteria.
b) Why for pour plate method is carried out in pharmaceutical microbiology?
c) Mention the various components of nutrient broth.
d) Draw a neat flow diagram of aseptic area.
e) Differentiate between antiseptics and disinfectants.
f) Name any four Gram positive and Gram negative bacteria.
g) Write down the various steps involved in 'assessment of a new antibiotic'.
h) Define HEPA and mention its efficiency.
i) Give few examples of preservatives used in pharmacy.
j) Name four different methods for quantitative measurement of bacterial growth.

Part-II

Q2 **Focused-Short Answer Type Questions- (Answer Any Seven)** **(5 × 7)**

Discuss briefly on the followings :

a) Bacterial growth curve.
b) Sterility indicators used in pharmacy.
c) Gram's staining.
d) Identification of bacteria using IMVIC tests.
e) Classify clean area according to ISO guideline.
f) Differentiate between light and Electron microscopy.
g) Classification and mode of action of various types disinfectants.
h) Factors influencing disinfection.
i) Replication of virus.

Part-III

Long Answer Type Questions (Answer Any Two)

Q3 Define sterilization. Discuss in details about the principle, procedure, merits, demerits and applications of physical method of sterilization. **(10)**

Q4 Discuss briefly about the principles and various methods of microbiological assay for antibiotics. **(10)**

Q5 Differentiate between prokaryotes and eukaryotes using various features of them. **(10)**

Q6 What is cell culture and mention its importance? Explain in details about the general procedure for cell culture. **(10)**

Part-I

Q1

a) The various nutritional requirements for Bacteria are-

* Water
* Carbon
* Nitrogen and Sulphur
* Oxygen
* Organic Growth factors

b) The pour plate Method is also known as the cylinder plate Method or Agar Diffusion Bioassay is a classical microbiology assay used in pharmaceutical microbiology to determine the potency (antimicrobial activity) of antibiotics and to ensure the quality control of antibiotic preparations.

c) The nutrient broth is a simple, general-purpose liquid medium used in pharmaceutical microbiology and other microbiological labs to support the growth of a wide range of non-fastidious microorganisms.

Components of Nutrient Broth are -

* peptone
* Beef extract
* Sodium chloride
* Distilled water
* PH

h) HEPA stands for high-efficiency particulate air filter. It is an advanced air filter that can remove extremely small particles from the air. They are able to capture at least 99.97% of airborne particles as small as 0.3 microns.

i) Common preservatives in pharmacies include Paraben, Sorbic acid, Sodium benzoate, benzyl alcohol, benzalkonium chloride and Phenoxyethanol.

e) Antiseptics

(i) Antiseptics are used on living tissues to kill or prevent the growth of microorganisms.

(ii) It does not cause any harm to the living tissues.

(iii) Antiseptics usually kill all microorganisms.

ex- 0.2 % Solution of Phenol.

Disinfectants

(i) Disinfectants are used on non-living objects to kill the micro-organisms

(ii) They are harmful to the living tissues and cannot be applied to the skin.

(iii) Disinfectants do not necessarily kill all microorganisms.

ex - 1% Solution of Phenol.

f) Gram +ve bacteria —

(i) Staphylococcus aureus.
(ii) Streptococcus pneumonia
(iii) Staphylococcus epidermidis
(iv) Streptococcus mutans.

Gram —ve bacteria —

(i) ~~Salmonella~~ Salmonella typhi
(ii) proteus vulgaris
(iii) Escherichia coli
(iv) Proteus mercabilis

Part - II

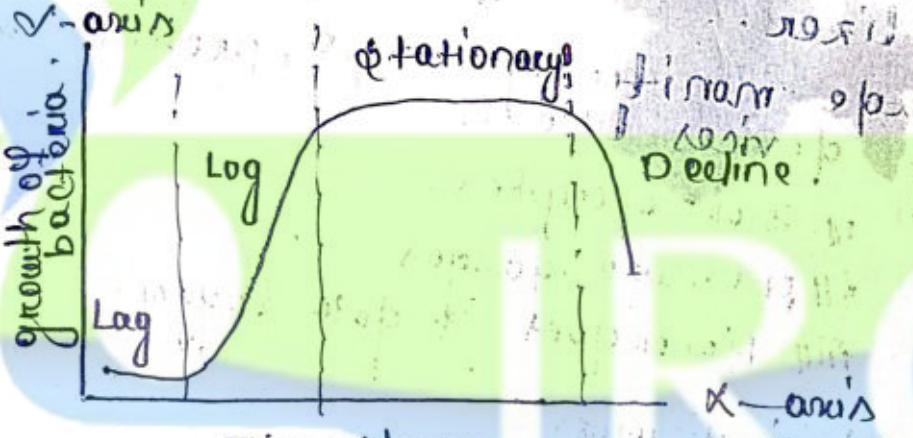
Q. Bacterial growth curve.

Ans Bacterial growth curve.

When a graph plot b/n the growth of bacteria & time phase is called 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, their are no growth of bacteria. because culture media is just prepared, so upto 2 hrs 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 germination of bacteria. they reproduce in fast rate & bacteria increase.

3) Stationary phase - In this phase, Growth of bacteria stop, & it may be stationary.

4) Decline phase - Also known as death phase, In this phase, bacteria start dying & no. of bacteria is more than nutrition medium.

b) Sterility indicator used in pharmacy

Ans:- Sterility indicators -

It is essential that strict controls are products to be labelled sterile. Such control much then ensure.

→ Monitoring of the sterilizatn process can be achieved by the use of physical, chemical or biological indicators of the sterilizatn performed.

(1) physical indicators :-

→ measurement of physical parameters of the sterilizer.

→ include monitoring temp^r, pressure & time using devices like.

i) thermocouples

ii) pressure gauges

iii) Recorders & data loggers.

2) Biological indicators :-

→ Contain highly resistant bacterial spores.

→ After sterilizatn, the spores are incubated in a growth medium to check if any survive.

Ex:- (1) Geobacillus stearothermophilus spores.

(2) Bacillus atrophaeus spores.

3) chemical indicators :-

Chemical monitoring of a sterilizatn process is based on the ability of heating steam sterilant gas & ionizing radiatn to alter the chemical or physical characteristics of a variety of chemical substances.

Ex:- (i) Browns tubes

(ii) witness labels

Q) Gram staining.

Ans- Gram staining-
→ In this staining we identified that, bacteria is grame -ve & gram +ve.
→ This staining performed by 2 or more dye.

Procedure-
→ Firstly take glass slide, cover slip, inoculatn loop, culture media microscope.
→ Now, wash all there with ethanol soln. then allow for drying, after drying put all there under flamming for complete sterilization.
→ Now, take inoculatn loop, streak into culture media in which bacteria attached on loop then streak inoculatn loop on glass slide
→ Now add some drops of crystal violet dyer as a indicator and allow to dry.
→ Now add gram iodine soln, which fix the crystal violet dye with cell wall
→ Now, wash the slide with ethanol & Acetone soln.
→ Now add few drops of safranine dye on surface of slide & allow to dry
→ Then observe it on microscope.

observatn
→ If purple color obtained then bacteria is gram +ve.
→ If Red color is obtained then bacteria is gram -ve.

Q// Identification of bacteria using IMViC test.

Ans- IMViC test

→ This is the biochemical series of test which is performed for differentiate into enterobacter.

→ eg- enterobacter coli.

I - Indole test
M - methyl Red test
Vi - Voges utilisation test
C - citrate utilisation test

1// Indole production test -

Many bacterial species, which posses enzyme tryptophanase.

→ The test is primarily used in microbiology for the identification of bacteria, particularly to distinguish both members of the family Enterobacteriaceae.

2// methyl Red test -

→ The methyl Red test is a biochemical test used to determine the ability of an organism to form stable mixed acids through glucose fermentation.

3// Voges - proskauer test -

→ It is a biochemical test used to detect the ability of an organism to produce Acetoin through glucose fermentation.

4// citrate utilization test -

→ It is a biochemical test used to determine if an organism can utilize citrate as only source of carbon & ammonium ions as its only nitrogen source.

e. Classify clean area according to ISO guideline.

- A cleanroom is defined as a room in which the concentration of airborne particles is controlled.
  (it may be controlled by HEPA filter and airlocked entry system)
- It established by measurement of the number of particles (airborne) with size 0.5 μ or greater, that are contained in $1 ft^3$ (1 cubic foot) of sampled area.
- According to US federal standard Association classification of clean room
  - Class 100 (Standard applicable to aseptic areas)
  - Class 1000
  - Class 10000
  - Class 1,00,000
  - this classification is used in pharmaceutical industry
  - Class 100 means that not more than 100 particles per cubic foot of size 0.5 μm or greater, shall be found in measured area
  - some other classes measured, according to point 2.

f) Differentiate between light and Electron microscopy.

Light microscope	Electron microscope
1. Light is the illuminating source.	1. The beam of electrone source.
2. Specimen preparation takes usually few minutes to hours. Live or dead specimen may be seen.	2. Specimen preparation takes usually takes a few days. only dead or dried specimen are seen.
3. Condenser, objective and eye piece lenses are made up of glasses	3. All lenses are electro magnetic.
4. Specimen is stained by coloured dyes.	4. Specimen is coated with heavy metals in order to reflect electrons.
5. It has low resolving power (0.25 µm to 0.3 µm). it has a magnification of 500X to 1500X.	5. It has high resolving power (0.001 µm), about 250 times higher than light microscope. it has a magnification more than 100,000X
6. Vacuum is not required.	6. Vacuum is essential for its operation.
7. Image is seen by eye through ocular lens.	7. Image is produce on fluorsent screen or photographic plate.

g) Classification and mode of action of various types disinfectants.

Disinfectants:- Disinfectants are antimicrobial agents that are applied to non-living

object to destroy microorganism, and the process is known as disinfection.

- Chemical agents are most commonly used as disiufectents. These chemical agents are classified as follow :-

i) Acids and alkalies :- The germicidal efficiency of acids is propational to the hydrogen (H^+) ion concentrations of their solutions. they produce toxic effects which dicreose or kill bacteria (micro organism)

eg. Hydrochloric acid, sulphuric acid, etc.

ii) Halogens :- The germicidal action of helogens and its compounds is due to formation of hypochlu-ous acid (or other acids) when free halogens reacts with water.

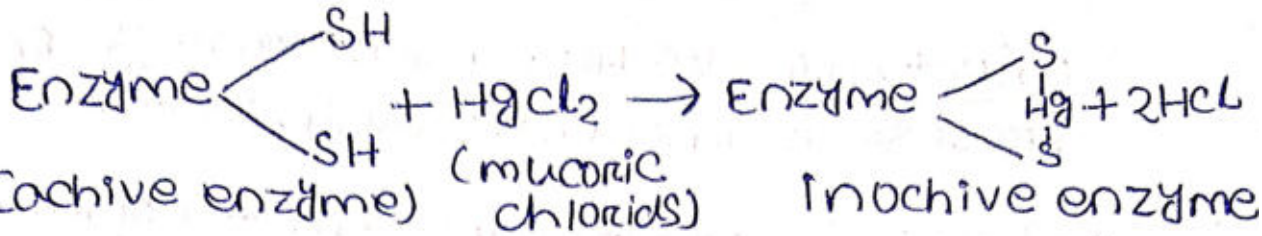
eg:- chloriul, Iodine, Bromine, flourine act as gennicidal.

$$Cl_2 + H_2O \longrightarrow HCl + HClO$$

(chlorine) (Hypochlorous acid)

iii) Heavy metals :- The most widely used compounds of heavy metal are those of mercury, Silver and copper.

- Heavy metals and their compounds act antimicrobiall by combining with cellwar proteins.



iv) Phenol and its derivatives

v) Alcohols

vi) Aldehydes

vii) Dyes

viii) Detergents and Soaps

ix) Quaternary ammonium compounds

h) factors influencing disinfection.

there are some following factors, which affect the action of disinfectants :-

i) Effect of temperature

ii) Effect of PH

iii) Effect of Dilution

iv) Effect of Surface activity

v) Effect of ~~surface of~~ interfering agent

i) Effect of temperature :-

If the temperature of the disinfectants is increased, the effectiveness of the disinfectante also increases.

ii) Effect of PH :-

The optimal bacterial growth take place in the range of 6-8, on either side of this the rate of growth dicliness. if we provide some PH, which bacteria need to grow, then

bacteria grow and disinfectants become less effecbe.

So, always provide different pH disiufetants to decline the growth of bacteria (microorganism)

iii) Effect of dillution / conciutrations:-

At higher concentration the drags acts as bactericidal, while on dilution the dug act as bact-eriosatic.

So, concentration of disinfectrent ↑ = effechiveness ↑ - But also used in limit.

iv) Effect of surface activity:-

The addition of low concenpation of surface active compounds may potentiate the biological effect of an antibocterial agent,

eg. Phenols are more active in the presence of soaps.

i) Replication of virus.

Viruses replicant inside host cells through the following steps:

1. Attachment - The virus binds to specific receptors on the host cell surface.
2. Entry - The virus or its genetic material enters the host cell by fusion or endocytosis
3. Uncoating - The viral capsid is removed, releasing the viral genome into the host cell.

4. Replication and protein synthesis - The viral genome is replicated and viral proteins are synthesized using host or viral enzymes.

5. Assembly and Release - New viral particles are assembled and released from the host cell by lysis (non-enveloped viruses) or budding (enveloped viruses), ready to infect new cells.

Q3 Define "sterilization. Discuss" in details about the Principle, procedure, merits, demerits and applications of Physical method of sterilization.

Sterilization

- It is a process in which remove or kill of all types of microorganism from the surface of all non-living or living things.
- It is an essential stage in the processing of any product use for parental administration internal organ.

Method of sterilization

1. Physical method
2. Chemical gaseous method
3. Radiation method
4. Mechanical method

Physical method

In this method, heat apply on the surface then proteins of bacteria are denatured and oxidised at high temp. and it kill.

- It is 2 types (a) Dry heat sterilization.
  (b) Moist heat sterilization.

(a) Dry heat sterilization

Heat is the most reliable and rapid method of sterilization. The killing effect of dry heat is due to protein denaturation oxidative damage and the toxic effect of

elevated levels of electrolytes.

· HOT AIR OVEN :-

This is the most widely used method of sterilization by dry heat.

Principle :- In Hot air oven, maintain the high temp. so the protein of bacteria and denatured and oxidised and the bacteria kills.

Procedure

- Non-heat labile substances put inside it.
- Normally the spaus of bacteria as well as vegetative form of all microorganism are killed in two hours at a temp. of 160°C.
- The oven must be allowed to cool slowly for about two hour before the door is opened, since the glass ware may get cracked by sudden cooling.
- Relation between temprature & time.

for hot air seen

Temprature (°c)	Time (minutes)
170°C	60min
160°C	120 min
150°C	150 min
140°C	180 min

Merit

- simple and easy in use.

Demerit :-

- Not suitable for surgical dressing, rubbers plastic volatile and heat labile substance.

Application

- Used to sterilize, Glass wall. Forceps, scalpels, Scissors, spatula etc.
- Also For some pharmaceutical substances such as glycerin, Fixed oil, liquid paraffin, propyline glycol etc.

(b) Moist heat sterilization :-

In this process, microorganism are killed by hot water or steam.

AUTOCLAVE

Principle - In which, saturated steam is procedure Under pressure.

So, Pressure increase in a closed vessels increase temprature properctionality.

And then high pressure is used to kill the microorganism.

Procedure - The water is filled in autoclave and material is to be sterilization is load inside the autoclave.

- Now maintain the temprature and pressure For sterilization, according to their need:-

Temp.(°c)	steam pressure (lb/sq.inch	Holding time (min.)
115 - 118	10	30
121 - 124	15	15
126 - 129	20	10
135 - 138	30	3

- After put the material, lid is closed discharge tap is opened and safety value is adjusted to the required pressure.

- When air bubbles stop omitting from the discharge tap is opened and safety; it indicates all the air removed from inside the autoclave.
- Then, close the discharge tap. Now, steam pressure rises inside and when it reaches the desired set level, safety value opens and excess steam escaps.
- After the holding time, the heating is stopped and allowed to cool the autoclave.
- Now, air is allow to remove from the autoclave by open discharge tap.
- Now, finally Lid is opened and the sterilized articles are removed.

Merits

- It is very effective
- It provide greater lethal action of moist heat.

Demerit

- Not suitable for anhydrous material such as powders, oils, fats etc.

Applications:-

- It is used for sterilization bacteriology media, heat stable liquid, saline solution heat resistant equipments and instruments, glasswares, filters, rubber producer etc.

Q. Differentate between prokaryotes and eukaryote using various features of them.

characterstics	Prokaryotic	Eukaryotic
1. Golgi Apparatus	Absent	Present.
2. Metabolism	Anaerobic/aerobic	Aerobic
3. ~~Metabolism~~ Locomotion	Rotating flagella & gliding movement.	undulating; cilia are absent amoeboid movemen
4. Cell size	Generally 1 to 10 um in linear dimension	Generally 5 to 100 um in leaner dimension.
5. Cellular structure	Unicellular	multicellular
6. Cell wall	Complex structure with Peptidoglycan,	Absent or mad up of Chitin
7. Plasma memb.	Present, generally no sterols.	present with sterol.
8. membrane bound Nucleus	Absent	Present
9. DNA	circular DNA in cytoplasm.	long, linear DNA bounded in nuclear envelope
10. Plasmid DNA	present	Absent.
11. Histones	Absent	present.
12. RNA & protein	RNA & protein synthesized in some compartment.	RNA synthesize in Nucleus, protein synthesi -zed in cytoplasm
13. Membrane bound organells	Absent	Present
14. Ribosomes	70s type	80s type
15. Lysosomes	Absent	Present
16. Flagella	Consist of 2 protein	consist of tubes multiple protei

17.	Pili	Present	Absent
18.	Site For cellular respiration	Cell membrane	Mitochondria
19.	Cell division	Binary Fission	Mitosis
20.	Sexual Reproduction	Conjugation	Meiosis
21.	Examples	Bacteria, Archaea	Fungi, Alage, Pla Protozoa, Animals

Q.4) Discuss briefly about the principles and various methods of microbiological assay for antibiotics?

(6) what is cell culture & mention its imp? explain in details about the general procedure for cell culture?

Ans:→ cell culture is the process of growing & maintaining cells outside their natural environment. under controlled laboratory condⁿ. it involves providing cells with suitable nutrients. temp^{n}. pH, gases (CO_2 & O_2). & a sterile environment.

Application of cell cultures in pharmaceutical industry & search

there are ~~vabes~~ various application of cell cultures:-

(1) model system :- It is used as a model system in research that helps us to study in basic cells biology, effects of substances on cells etc.

(2) production of vaccines :- cells are used to produces vaccines. e.g. for hepatitis, rabies, chicken pox vaccines.

procedure of cell culture :→

A pieces of tissue from the organism is usually quite complex & it comprises it contains connective tissue cells, variety of blood cells & reticuloendothelial cells.

- the 1st cells suspension is isolated & then inoculated into a new culture vessels along

with fresh medium (primary cell cultures).

- Stages for cells cultures:-

(i) isolation of the tissue (ii) disaggregation (iii) seeding the culture into culture vessels.

(i) isolation of the tissue :→ The explant from an excised (cut surgically) portion of the body of an animal is used for the culture of animal cells in a suitable nutrient medium.

- Animals are mice, rabbits, guinea pigs etc.
- organs from which cells are to be isolated are surface sterilised with 70% alcohol & then removed aseptically.
- The tissue isolate from explant is either stored in freeze or used immediately.

(2) Disaggregation of tissue :→ A primary cell culture is obtained by disaggregating the tissue mechanically, enzymatically or by use of chelating agent (EDTA)

(3) seeding the culture into the culture vessels → The dissociated (primary) cells grow well when seeded on culture plates at high density.