JRG College of Pharmacy

JRG COLLEGE OF PHARMACY

UNIVERSITY SOLVED QUESTION WITH ANSWER

Year	:	**2021-22**
Subject	:	**Ph. Microbiology**
Subject Code	:	**BP303T**
Subject In-Charge	:	**Mr. Biswajit Biswal**

[redacted]

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

Total Number of Pages : 01

B. Pharm
BP303T

3rd Semester Regular / Back Examination: 2021-22
PHARMACEUTICAL MICROBIOLOGY
BRANCH: B. Pharma
Time : 3 Hour
Max Marks :75
Q.Code : OF712

Answer Question No.1 (Part-I) 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 :** **(2×10)**

a) Write down the nutritional requirements for bacteria.
b) Why for spread plate method is carried out?
c) Write the composition of nutrient broth.
d) Draw a flow diagram of aseptic area.
e) Give few examples of preservatives used in pharmaceutical formulations.
f) Differentiate disinfectants and antiseptics.
g) What is Acid fast bacteria and give an example of it?
h) Write down the different steps involved in 'assessment of a new antibiotic'.
i) Mention the full form and efficiency of HEPA?
j) Name any four Gram positive bacteria.

Part-II

Q2 **Focused-Short Answer Type Questions- (Answer Any Seven)** **(5×7)**
Discuss briefly on the followings:

a) Factors influencing disinfection
b) Bacterial growth curve
c) Electron microscopy
d) Classification and mode of action of disinfectants
e) Application of cell culture in pharmaceutical industry
f) Sterility indicators
g) Identification of bacteria using IMVIC tests
h) Gram's staining
i) Classification of clean area according to ISO guideline

Part-III

Long Answer Type Questions (Answer Any Two)

Q3 Differentiate between prokaryotes and eukaryotes using various features of them. **(10)**

Q4 What do you mean by sterilization? Discuss about principle, procedure, merits, demerits and applications of physical method of sterilization. **(10)**

Q5 Discuss briefly about the principles and methods of different microbiological assay. **(10)**

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

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Pharmaceutical microbiology 2021-22

(1) write down the nutritional requirement of bacteria?
(A) Bacteria require macro nutrients and micro nutrients
(i) macro nutrient :- carbon, nitrogen, sulphur, phosphorous, hydrogen, oxygen.
(ii) micro nutrients :- Trace element like zinc, copper, manganese and vitamins for enzyme function and metabolism.

(2) Why for spread plate method is carried out?
(A) The spread plate method is carried out to isolate and count viable microorganism present in a sample by spreading a diluted culture even on surface of a solid agar medium, allowing individual colonies to grow for enumeration and study.

(3) write the composition of nutrient broth?
(A) peptone - 5.0g (source of amino acid and nitrogen)
Beef extract - 3.0g (source of vitamins and minerals)
Sodium Chloride - 5.0g (maintains osmotic balance)
Distilled water - 1000ml
PH Adjusted to - 7.0 before sterilization.

(4) Draw a flow diagram of aseptic area?

Flow diagram of aseptic area.

e) Give few examples of preservatives used in pharmaceutical formulations?

A) Examples are :- Benzyl alchol
- methyl paraben
- propyl paraben
- phenol
- chlorocresol
- Benzalkonium chloride.

f) Differentiate disinfectant and antiseptics?

A) disinfectant	Antiseptic
- used on nonliving surface.	- used on living tissue
- usually stronger and more concentrated	- milder, suitable for skin application
- Phenol, lysol, formaldehyde	- Dettol, iodine, Alchol

g) What is the Acid fast bacteria and give an example of it?

A) Acid fast bacteria are those that retain the primary stain (carbol fuchsin) even after treatment with acid alchol, due to the presence of mycolic acid in their cell wall.
Example - mycobacterium tuberculosis.

h) write down the different steps involved in assesment of a new antibiotics?

A) Screening :- Testing microorganism or compounds for antibacterial activity.
Isolation and identification :- identifying the active antibiotic compound.
Invitro testing :- Determing spectrum and potency against various bacteria.
Toxicity testing - Checking saftey in animals or cell culture.
Clinical trials :- Evaluating saftey and effectiveness

in humans.

c) mention the full forms and efficiency of hepa ?

A) full form of hepa - high efficiency particulate Air.
Efficiency :- hepa filters can remove 99.97% of particles having a size of 0.3 micrometers (μm) or larger

J) Name any four gram positive bacteria ?

A) four gram positive bacteria
1) staphylococcus aureus
2) streptococcous pyogenes
3) Bacillus subtilis
4) Clostridium tetani

Part II

2a) factors influencing disinfection ?

A) Concentration of disinfectant :- Higher concentration usually increase the rate of disinfection upto an optimum level.

Contact time :- longer exposure allows more effective killing of microorganisms.

Temperature and pH :- Higher temperature and suitable Ph enhance the activity of many disinfectants.

Type and number of microorganism :- some microbes are more resistant than others larger population need more time.

Presence of organic matter :- Blood, pus or dirt can reduce the effectiveness of disinfectant by reacting with them.

b) Bacterial growth bacteria?

1) The bacterial growth curve is a graphical representation of the growth of a bacterial population time under controlled condition.
it has four distinct phase.

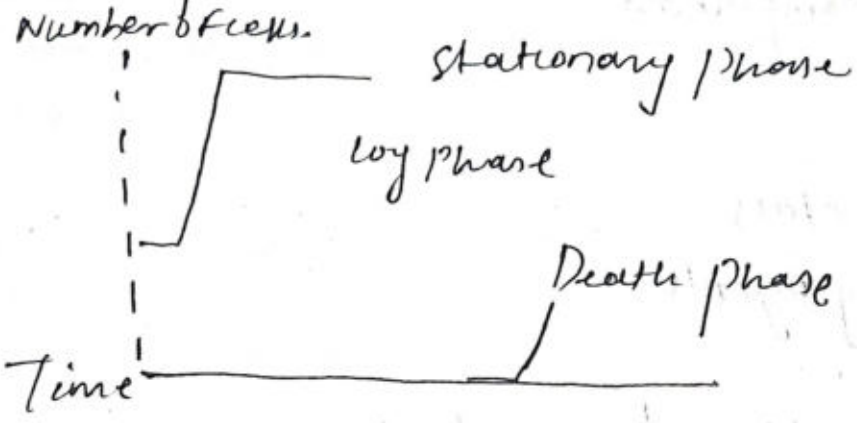
lag phase : Bacteria are metabolically active but not dividing.
- cells are adapting to the new environment synthesizing, enzyme and repairing damage.
- Flat portion at the beginning.

log phase : Rapid cell division occurs population double at a constant rate.
- maximum growth rate.
Nutrients are abudant.
Cells are most susceptible of antibiotics.
- step upward slope.

Stationary phase :
- growth rate slows and the number of new cells equals to the number of dying cells.
- Nutrient depletion, waste accumulation.
- Horizontal Line

Death phase :
- number of dying cells exceeds new cells formed.
- toxic waste accumulation

- Downward slope



C) Electron microscopy :-

A) Electro microscopy is a technique that uses a beam of electrons instead of light to produce highly magnified images of specimens. it provide much higher resolution than light microscopy because electrons have much shorter wavelength than visible light.

Types of electron microscopy

- Transmission electron microscopy
- Scanning electron microscopy

Advantage

- Very high magnification and resolution
- can reveal ultra structural details

disadvantage

- Expensive instruments and maintainence.
- complex sample preparation

D) Classification and mode action of disinfectan

A) Disinfectant Classify based on chemical and nature Mechanism of action

1. Phenolic compounds
2. Halogens
3. Alchols
4. Heavy metals
5. oxidizing agents
6. Aldehyde.

modes of action disinfectant :-

- cell membrane disruption
- protein denaturation
- oxidation of cellular components
- binding to nucleic acid enzymes

e) Application of cell culture in pharmaceutical industries ?

A) Cell culture is the growth and maintainance of cell under controlled condition outside the organism. In the pharmaceutical industry it is widely used for drug development.

- production of bio pharmaceutics
- Drug screening and development
- Toxicity testing
- Gene therapy and genetic engineering
- Tissue engineering and regenerative medicine
- vaccine development

f) Sterility indicators ?

A) Sterility indicators are tools or device used to confirm that a sterilization process has been effective in killing all viable

microorganisms.
- They are widely used in hospitals, pharmaceutical production.

Type of sterility indicators

- physical indicator :- Ex :- Thermometer, pressure gauge and timers.

chemical indicator :- Ex :- Autoclave tape, chemical strips

Biological indicator :- Bacillus, subtetis.

Q) gram's staining ?

A) gram staining is a differential staining technique used to classify bacteria into gram positive and gram negative based on their cell wall structure.

Principle

- gram positive bacteria - Thick peptidoglycan layer → retain crystal violet → purple/blue
- gram negative bacteria - Thick peptidoglycan + outer membrane → do not retain crystal violet → take counter stain → pink red.

Reagent used

- Primary stain - crystal violet
- mordant - iodine solution
- Decolorizer - Alchol aceton
- Counter stain - safranin.

Part - III

(4) Difference between eukaryotes and prokaryotes.

prokaryotes	eukaryotes
- Prokaryotic cells doesn't poses nucleus and membr are - bound organells	- Eukaryotic cells posses membrane bound organells including the nucleas.
- Normally 0.2 to 2μm in diameter	- Normally 10 to 100μm in diameter.
- Have no true nucleus no nuclear membrane	- Consist of a true nucleas with nuclear membrane and nucleoli
- Consist of single circular DNA molecule in the Nucleoid	- Consist of multiple linear Chromosomes in the nucleas
- membrane bound organells are absent	- membrane bound organells are present.
- flagella are made up of tub proteins	- Some cells without cell wall contain flagella
- mostly made up of peptido glycans	- made up of cellulose and chotin and pectin
- Carbohydrates and sterols are not found in plasma membrane.	- Carbohydrates and sterols serves as receptor on the plasma membrane
- Small in size	- Large in size
- Cell division occurs through binarry fission.	- Cell devision takes place through mitosis.

b) What do you mean by Sterilization? Discuss about the principle, procedure, merits, demerits of physical sterilization?

A) Sterilization is a process by which all forms of microbial life - including bacteria, viruses, fungi, and spores are completely destroy or removed from any surface, liquid or equipment

principle of Physical Sterilization

physical method of sterilization works on the principle of denaturation or coagulation of microbial proteins, oxidation of cell components or destruction of cell membrane by heat radiation or filtration.

Type of physical Sterilization :-

Heat Sterilization :-

moist heat sterilization :- (Steam under pressure)

Example :- Auto clave

principle :- Steam under pressure increase temperature (121°C at 15 psi for 15-20 minutes)

uses - culture media, dressings.

Dry heat :-

Example - hot air oven

Principle :- oxidation of cell component at high temperature (160 - 180°C) for 1 to 2 hrs

uses - glass ware, oils, powders

Radiation Sterilization

- ionizing radiation :- gamma rays or electron beams destroy DNA.
- non-ionizing radiation :- UV rays cause thymine dimer formation in DNA
- used - disposable medical products, packaging materials

filtration

principle : physical removal of microorganism by passing liquid or air through a filter of pore size small enough (0.22µm)

used - for heat sensitive solutions (like antibiotics sera, vaccines)

merits

- No chemical residue remain.
- Effective and reliable when properly performed.
- Environmentally safe.

Demerits

- Not suitable for heat sensitive materials
- some methods require expensive requirement
- Filtration cannot removes virus sometime.

c) ~~Ex~~ Discuss about briefly about the method and principle of different microbial assay?

A) microbial assay is a biological method used to estimate the potency or concentration of antibiotics and other antimicrobial agents. by measuring their effect on the growth of microorganism under standardized condition.

principle

- The principle of microbial assay is based on the inhibition of the growth of the microorganism by antibiotic

methods of microbial assay

1) Cup plate method : The antibiotic diffuses from cups or discs into agar medium inoculated with a test microorganism.

- After incubation zone inhibition are measured.
- The size of the zone indirates the potency of antibiotics

Turbidimetric or broth method

- The growth of microorganism occurs in a liquid medium containing the antibiotics.
- The turbidity of the medium is inversly proportional to the antibiotic concentration
- measured spectrophotometrically.

End point method

- serial dilution of the antibiotics are prepared
- The lowest concentration that inhibits visible growth is recorded as the minimum inhibitory concentration.

Uses

- Determination of antibiotic potency.
- Standardization of antibiotic preparation.

END
Thankyou