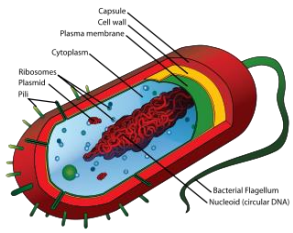
Introduction, scope and importance of microbiology. Prokaryotes and Eukaryotes (Lecture 1)

Scope and importance of pharmaceutical microbiology

1) Production of antibiotics
2) Production of enzymes, vaccines, bio-surfactants, alcoholic and other pharmaceutical products
3) Diagnosis of diseases and treatment
4) Sterile product preparation
5) Steroid biotransformation
6) Testing of pharmaceuticals
   b)Microbiological assays of antibiotics
   c)Evaluation of disinfectants, sterility test
   d)Antimicrobial preservative efficacy testing, Endotoxin testing

Prokariotic cell

Size is less than 5µm. Nucleus is primitive - Nuclear membrane & nucleolus is absent. Membrane bound cell organelles (mitochondria, endoplasmic reticulum, lysosomes) are absent. Cell wall of prokaryotic cell contains peptidoglycon. Ex.Bacteria, cyanobacteria



Eukaryotic cell

True nucleus is present. Nuclear membrane & nucleus is present. Membrane bound cell are present

Ex. Fungi, protozoa, plants, animals.

References -

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.

Ultra-structure and morphological classification of bacteria (Lecture 2)

Structure of bacterial cell- parts of bacterial cell

1. Cell Wall - Peptidoglycon is the major constituent of the cell wall

2. Cytoplasmic membrane- It is the layer lining the inner surface of cell wall

3. Cytoplasm-It is a suspension of organic, inorganic solutes in a water solution.

4. Cytoplasmic Inclusiions: Ex. Lipid granules, polyphosphate granules

5. Ribosomes - 70S type . They consist of 2 subunits of 30s and 50s

6. Nuclear material- It is ds DNA, covalently circular present openly in cytoplasm.

7. Endospores - Endospores are thick walled, highly refractile bodies, resistant to the heat, drying, staining and chemicals.

8. Flagella - long, slender, thin hair like cytoplasmic appendages, responsible for the motility

9.Pill or fimbriae - These are hair like microfibrils, about 0.5-7nm in diameter

10.Capsule - -It is the deposition of organic exopolymers on the cell wall .

MORPHOLOGICAL CLASSIFICATION OF BACTERIA

1. Cocci- spherical or oval cells Ex. *Micrococcus.*

2. Bacilli- Rod shaped cells Ex. *Bacillus anthracis.*

3. Curved bacteria-

a.Vibriod bacteria Ex. *Vibrio cholera.*

b.Helical bacteria Ex. *Spirillum*

4. Filamentous - Actinomycets- Ex. *Streptomyces Species.*

References -

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.

Nutritional requirements and culture media (Lecture 3)

Nutritional requirements of all living organisms are similar. Nutritional requirements of bacteria are as follows:

1) Source of energy: oxidation of chemicals - Chemotrophs / sunlight - Phototrophs

2) Source of electrons: Lithotrophs - Reduced inorganic/ organic compounds.

3) Source of nitrogen Nitrogen – Nitrogen gas/ Inorganic nitrogen, Organic nitrogen compounds – amino acids, proteins.

4) source of oxygen, sulphur, Phosphorus

5) Metal ions - Ca^{++}, Mg^{++}, K^{+}, Fe^{++}, Trace elements – Zn^{++}, Cu^{++}, Mn^{++}, Ni^{++}, Co^{++} , Mo^{6+},

6) Water: Nutrients must be present as aqueous solution for absorption by bacteria.

7) Vitamins - Most of the bacteria can synthesize their vitamins.

Raw material used for media preparation: peptone, meat extract, yeast extract, agar

References -

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.

Physical parameters for growth (Lecture 4)

1. Temperature

Microorganisms require suitable temperature for growth.

There are three cardinal temperatures characteristic of microorganisms:

- Minimum temperature
- Optimum temperature
- Maximum temperature

Bacteria are divided in to three groups on basis of temp. requirements:

1. Psychrophiles- able to grow at 0^0 c.

2. Mesophiles- able to grow at 25 to 40 ^{0} c.

3.Thermophiles- able to grow best at above 45^0 c.

2. Gaseous requirements of bacteria

Depending on oxygen requirement bacteria are classified as

1.Aerobic bacteria: Require oxygen for growth.

2.Anaerobic bacteria:Do not require oxygen for growth. *Ex- Clostridium spp.*

3.Facultatively anaerobic bacteria:Do not require oxygen for growth, but when present can use it for growth**.**

4.**Microaerophilic bacteria**: Require low concentration of oxygen.

3. pH requirement of bacteria

Most of the bacteria grow best around neutral pH, while some are acidis or alkaline pH

References -

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.

Growth curve of bacteria (Lecture 5)

It is the graph of log of number of viable cells verses time in hours. It shows following stages:

1) Initial stationary phase / Lag period

There is no increase in number of cells. It is time taken by cells to adjust with new environment. Synthesis of proteins ,enzymes, coenzymes occurs.

2) Logarithmic growth phase

Cell population increases exponentially. Generation time is constant & shortest.

Cell population is uniform with respect to chemical composition, metabolic activities, and relative concentrations of metabolites. Cell population is metabolically very active. It lasts for few hours.

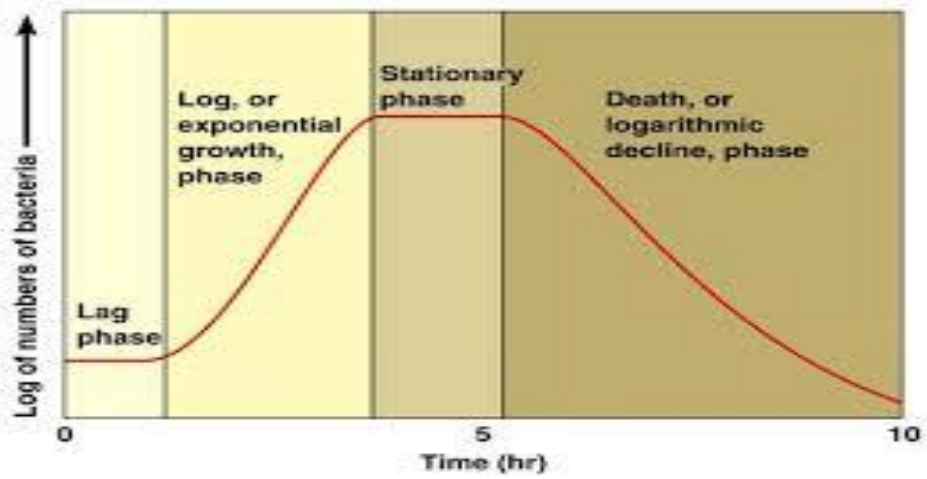
3) Maximum stationary phase

There is no increase in number of bacteria. Growth rate & death rate are equal.

4) Exponential death phase

Number of viable cells decrease exponentially.

Here bacterial population remains stationary. At very low level no. of new cells produced is equal to no. of cells die. For most of the bacteria this phase lasts for 3-4 days. .



References -

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.

2.Tortora, Funk, Case, Microbiology an Introduction, 10/Ed., Pearsons publication 2010.

Isolation of bacteria/ Pure culture technique (Lecture 6)

- Natural microbial populations are mixed cultures.
- To study properties and to prepare products from microorganisms ,they should be in pure culture.Mass of growth of cells containing only one type of cells is called pure culture.

Methods of isolation:

1) The streak plate technique: Dilution of bacteria on surface of solid nutrient media.

2)Pour plate technique; 1ml of dilution is added to tube of liquid (cooled) agar medium and mixed. Mixture is poured in Petri plate.

3) Spread plate techniqueSample is evenly spread on surface by sterile glass spreader.

4) Micromanipulator technique:

- Uses instrument – micromanipulator.
- Uses microscope in conjunction with micromanipulator.
- Single cell is picked up with micropipette.
- Pure culture is obtained from single cell. Clone.

References -

- 1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.
- 2.Tortora, Funk, Case, Microbiology an Introduction, 10/Ed., Pearsons publication 2010.

Preservation and maintenance of culture (Lecture 7)

Need for preservation:

- Laboratory and **research** work
- Production of **vaccines**, **antisera, fermentation**.
- **Standard organisms** for assay of antibiotics, vitamins and various tests

Aim of preservation:

- To maintain strains alive.
- To keep strains uncontaminated.
- To prevent any changes in their characteristics.

Methods of preservation:

- Periodic transfer to fresh media.
- Preservation by overlaying cultures with mineral oil.
- Lyophilization.
- Storage at low temperature / Cryopreservation
- Soil culture

References -

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.
2. Kokare C.R., Pharmaceutical Microbiology – Principles and Applications, 6/Ed., Nirali Prakashan, pune, India 2008.
3.Tortora, Funk, Case, Microbiology an Introduction, 10/Ed., Pearsons publication 2010.

Cultivation of anaerobes (Lecture 8)

Oxygen toxicity:

Free radicals are generated when oxygen is used

$O_2 + e^- \rightarrow O_2^-$ (superoxide synthase)

$2\ O_2^- + 2H^+ \rightarrow O_2^- + H_2O_2$ (superoxide dismutase)

$O2^- + H_2O_2$ (chelated iron) $\rightarrow O_2 + OH^- + OH\ °$

Aerobic bacteria produce **catalase, peroxidase** & eliminate free radicals

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

Anaerobic bacteria do not produce these enzymes and cannot eliminate free radicals. Hence in presence of oxygen they are killed.

Methods of cultivation:

1.Prereduced media: Reducing agent like sodium thioglycollate, cystein is added.

2. Anaerobic chamber: It is a plastic glove box. It is filled with H2, N2 and CO2. It contains palladium catalyst for reaction of oxygen in air with hydrogen and is removed.

3. Anaerobic Jar: It is thick walled glass or steel cylindrical jar: Lid can be clamped to jar by screw. Commercially available CO2 and H2 generating system is added to jar. Generated H2 reacts with oxygen present in jar with help of palladium catalyst and anaerobic conditions are created.

References -

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.

Quantitative measurement of bacterial growth (Lecture 9)

MEASUREMENT OF BACTERIAL GROWTH

The term growth as employed in bacteriological refers to the magnitude of the total population. Growth can be determined by various techniques

1. Determination of number of cells.

Direct method:-

(a) Microscopy count (b) Breed's method

(c) Proportional count method (d) Electronics counters

Indirect method

(a) The plate count (colony count) (b) Membrane-filter count

2.Determination of cell mass.

Direct method:- .

(a) Dry weight measurement (b) Measuring of cell nitrogen

Indirect method:-

Turbidity measurement

3. Determination cell activity.

By indirect method

By relating the degree of biochemical activity to the size of the population.

References -

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.

Study of different types of phase constrast, dark field and electron microscopy. (Lecture 10)

The Phase-Contrast Microscope

- It enhances the contrast between intracellular structures having slight differences in refractive index.It is an excellent way to observe living cells.
- A phase-contrast microscope splits a beam of light into 2 types of light, direct and refracted (reflected) and brings them together to form an image of the specimen.
- Where they are "in-phase" the image is brighter, where they are "out of phase" the image is darker, and by amplifying these differences in the light, it enhances contrast

The Dark-Field Microscope

- It produces a bright image of the object against a dark background.
- It is used to observe living, unstained preparations.
- It uses dark field condenser which has stopper disc below the condenser. All central light rays are blocked.
- It allows only oblique, peripheral rays to reach object.

Electron Microscopy

- Beams of electrons are used to produce images
- Wavelength of electron beam is much shorter than light, resulting in much higher resolution
- It is capable of much higher magnifications and has a greater resolving power than a light microscope, allowing it to see much smaller objects in finer detail.
- All electron microscopes use electromagnetic and/or electrostatic lenses to control the path of electrons.

References -

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.

Simple, Gram's staining (Lecture 11)

Simple staining

Staining of microorganisms is one of the most common methods of studying morphology. Simple staining is done by application of basic stain. Basic stains after ionization produce colored cation. Surface of bacteria is negatively charged. Positively charged ions of basic dye form bond with negative charges on bacterial surface and coat bacterial surface. The stain is retained even after subsequent washing with water for removal of excess dye from slide.

Gram Staining

Christian Gram introduced this technique of differential staining which is useful in distinguishing bacteria as Gram positive and Gram negative. Cell wall of Gram-positive bacteria is thick. Its peptidoglycan content is high and is highly cross-linked. Therefore it is less porous. It has low lipid content. Whereas cell wall of Gram-negative bacteria is thin. Its peptidoglycan content is low and is less cross-linked. Therefore it is more porous. Lipid content is high. During decolourization treatment ethanol is applied. Ethanol is a lipid solvent and removes lipid from cell wall of Gram-negative bacteria. Therefore porosity increases. In Gram staining insoluble precipitate of crystal violet–iodine complex is formed in cell wall .In ethanol treatment CV-I complex comes out of cell wall of Gram negative bacteria as pores in cell wall are large. Therefore they become colourless and later take secondary stain and appear pink .In Gram positive bacteria pore size decreases as ethanol is dehydrating agent which brings about shrinkage of cell wall. Therefore CV-I complex is retained in cell wall and bacteria appear violet.

References:

Anathnarayan R. and Panikar C. K. J., (2000). Textbook of Microbiology, 6/Ed., Orient longman.

Acid fast staining (Lecture 12)

Acid-fast stain, first introduced by Dr. Paul Ehrlich, also known as the Ziehl–Neelsen staining, is a bacteriological stain used to identify acid-fast organisms, mainly Mycobacteria.

It is named for two German doctors who modified it: the bacteriologist Franz Ziehl (1859–1926) and the pathologist Friedrich Neelsen (1854–1898).

Mycobacterium tuberculosis is the most important of this group because it is responsible for tuberculosis (TB).

Other important Mycobacterium species involved in human disease are *Mycobacterium leprae*, *Mycobacterium kansasii*, *Mycobacterium marinum*, *Mycobacterium bovis*, *Mycobacterium africanum* and members of the *Mycobacterium avium* complex.

It can also be used to stain a few other bacteria, such as *Nocardia*..

Acid-fast organisms like Mycobacterium contain large amounts of lipid substances within their cell walls called mycolic acids.

These acids resist staining by ordinary methods such as a Gram stain.

Initially, carbol fuchsin stains every cell. When they are de-stained with acid-alcohol, only non-acid-fast bacteria get de-stained since they do not have a thick, waxy lipid layer like acid-fast bacteria. When counter stain is applied, non-acid-fast bacteria pick it up and become blue (methylene blue) or green (malachite green) when viewed under the microscope. Acid-fast bacteria retain carbol fuchsin so they appear red.

References:

1. Anathnarayan R. and Panikar C. K. J., (2000). Textbook of Microbiology, 6/Ed., Orient longman.

IMViC Tests (Lecture 13)

The **IMViC** tests are a group of individual tests used in microbiology lab testing to identify an organism in the coliform group.

Indole test

Under the action of enzyme tryptophanase, tryptophan is converted to an Indole molecule, pyruvate and ammonium. The indole is then extracted from the broth by means of xylene. To test indole production, Kovac's reagent is used.A positive result is indicated by a pink/red layer forming on top of the liquid.

Methyl Red and Voges – Proskauer Test

These tests both use the MRVP broth for bacterial growth.

The methyl red test detects production of acids formed during metabolism using mixed acid fermentation pathway. The pH indicator Methyl Red is added and a red color appears at pH's lower than 4.2, indicating a positive test (mixed acid fermentation is used).

The VP test uses alpha-naphthol and potassium hydroxide to test for the presence of acetylmethylcarbinol (acetoin), an intermediate of the 2,3-butanediol fermentation pathway. After adding both reagents, the tube is shaken vigorously then allowed to sit for 5-10 minutes. A pinkish-red color indicates a positive test, meaning the 2,3-butanediol fermentation pathway is used.

Citrate test

This test uses Simmon's citrate agar to determine the ability of a microorganism to use citrate as its sole carbon source. The citrate agar is green before inoculation, and turns blue as a positive test indicator, meaning citrate is utilized.

STERILISATION BY DRY AND MOIST HEAT (LECTURE 14)

Sterilization –It is the process of freeing an article from microorganisms including their spores.

Dry heat sterilization

Dry heat kills microorganisms by oxidation of chemicals.

1. Hot air oven: utilizes dry heat to sterilize articles. A holding period of 160ºC for 1 hr is desirable. There is a thermostat controlling the temperature. Double walled insulation keeps the heat in and conserves energy.

Uses: To sterilise Forceps, Scissors, Scalpels, Swabs, Pharmaceuticals products like Liquid paraffin, dusting powder, fats and grease.

2. Flaming - Inoculation loop or Wire, the tip of Forceps and spatulas

3.Incineration- Animal carcasses and pathological materials.

Moist heat sterilization

Kills microorganisms by **coagulating** their proteins. Moist heat sterilisation is carried out with following methods

1. Steam at atmospheric pressure: Koch/Arnold's steamer – Tyndallisation. For media containing sugar and gelatin exposure of 100ºC for 20 min for 3 successive days is used

2. Steam under pressure: Autoclave.

Principle : Boling point of water increases with increase in pressure.

Sterilisation is carried out under by steam under pressure at 121º for 15 mnts. Moist heat kills microorganisms by coagulation of proteins. Penetration power of steam is high. It is more effective than dry heat.

Use : sterilization of nutrient media, aqueous injections, surgical dressings, ribber gloves

References:

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.

2. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

RADIATION STERILIZATION (LECTURE 15)

Energy is transmitted through space in form of Radiations .

Two types of radiations are used for sterilization

1. Ionising radiations

Energy content is more than 10 eV, short wavelength, penetration power is high. Ex. Gamma rays, X rays, Cathode rays.

X rays - Wave length = 1- 100 Angstrom. Lethal to microorganisms and higher forms of life, expensive, difficult to utilize

Gamma rays - Wave length = 0.01 – 1 Angstrom - shorter than X rays.

Obtained from radioactive isotope of cobalt. Energy content is higher than X rays.

Mechanism of action - Knock electron away from molecule and ionize them. Create free radicals. Free radicals cause intracellular damage.

Applications - Commercial sterilization of materials of considerable thickness or volume. Sterilization of packed medical material. Sterilize plastics Syringes, catheters, grease fabrics metal foils. Sterilization of packed food.

Non ionizing radiations Ultra violate radiations.

Wavelength – 150 – 3900 Angstrom. Penetration power is poor. Energy level is lesser than ionizing radiations.

Mechanism of action: Electron is excited to higher energy level and create excited molecule. Majorly absorbed by NA. Formation of pyrimidine dimer. Inhibition of DNA replication, mutation.

Application – Surface sterilization- Hospital operating rooms, Aseptic filling rooms,treatment of contaminated surfaces in dairy and food industries, of water.

STERILIZATION BY FILTRATION (LECTURE 16)

Filtration helps to remove bacteria from heat labile liquids such as sera and solutions of sugar, Antibiotics.

The following filters are used

1. Candle filter -Widely used for purification of water.

(a) Unglazed ceramic filter – Chamberland filter

(b) Diatomaceous earth filters – Berkefeld filter

2.Asbestos filters - Disposable single use discs, High adsorbing tendency, Carcinogenic. Eg: Seitz filter

3.Sintered glass filter -Prepared by heat fusing powdered glass particles of graded size, cleaned easily, brittle, expensive.

4.Membrane filters - Made of cellulose esters or other polymers

Uses - Water purification and analysis

Sterilization of heat sensitive material

and sterility testing

Preparation of solutions for parenteral use

5. HEPA filtes – High efficiency particulate air filters

These are 99.99% efficient in removing particles of size 0.3µm or larger

These are made up of fiber glass. They are used for sterilization of air

References:

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.
2. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

Chemical methods of sterilization (lecture 17)

1. Ethylene Oxide

Colorless, highly penetrating gas with a sweet ethereal smell. It is liquid at temperature below 10.8 ^{0}C. Highly flammable, high penetration power.

Mode of action of ethylene oxide

Alkylation of organic compounds such as enzymes and proteins. Free hydrogen atom in carboxyl, amino and sulfhydryl group is replaced by alkyl group.

Applications 1. Specially used for sterilizing heart-lung machines, respirators, sutures, dental equipments, books and clothing.

2. Sterilization of packed surgical material.

3. Decontamination of space ship components.

2. Beta propiolactone

Liquid at room temperature. B.P. – 155 ^{0}C.Vesicant and lachrymator. Not flammable. Lack penetration power. Carcinogenic properties.

Applications: Beta propiolactone is used in fumigation. For sterilisation 0.2% BPL is used.

3. Formaldehyde gas

Gas is obtained by heating formalin or paraformaldehyde. It is widely employed for fumigation of operation theatres and other rooms. Penetration power is limited. Require temperature 22 ^{o} C and relative humidity 60-80 %

References:

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.
2. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

Evaluation of the efficiency of sterilization methods. (Lecture 18)

D-value: of an organism is the time required in a given medium, at a given temperature, for a ten-fold reduction in the number of organisms.

Z-value: is a term used in microbial thermal death time calculations. It is the number of degrees the temperature has to be increased to achieve a tenfold (i.e. 1 $\log_{10}$) reduction in the D-value.

The z-value is a measure of the change of the D-value with varying temperature, and is a simplified version of an Arrhenius equation and it is equivalent to

$Z = 2.303\ RT\ T_{ref}/E.$

Z value is the temperature required for the thermal destruction curve to move one log cycle.

While the D-value gives the time needed at a certain temperature to kill 90% of the organisms, the z-value relates the resistance of an organism to differing temperatures. The z-value allows calculation of the equivalency of two thermal processes, if the D-value and the z-value are known.

F_0 value: is defined as the number of equivalent minutes of steam sterilization at temperature 121.1 °C delivered to a container or unit of product calculated using a z-value of 10 °C. The term F-value or "$F_{Tref/z}$" is defined as the equivalent number of minutes to a certain reference temperature (T_{ref}) for a certain control microorganism with an established **Z-value**

References:

2. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

Equipments employed in large scale sterilization.

(Lecture 19)

Sterilizers are special large scale sterilization equipments are used to make product free from live bacteria or other microorganisms.

Pharmaceutical and health care industries are more concerned about safety issues and thus sterilizers are essential to control microbial contamination

Different types of sterilization equipments use different technology and processes, according to application:

1. Moist steam sterilizer
2. Dry heat sterilizer
3. Ethylene Oxide sterilizer
4. CIP system
5. sterilization in place systems

For sterilization of medical waste, Steam sterilizers are cost effective to sterilize infectious waste and other material.

Various different large scale autoclaves that are used in pharmaceutical and biotechnology industries.

References:

1. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

Sterility indicators - lecture 20

1. Physical indicators
2. Chemical indicators
3. Microbial indicators

1. Physical indicators

A) Moist heat – thermocouple is inserted into coolest part of autoclave to measure inside temperature. Compared with - Master temperature record.

B) Dry heat - temperature record chart is compared with Master temperature record.

C) Radiation sterilization - Dosimeter exposed to radiations - dark

D) Gaseous sterilization - Measurement of temperature, humidity, gas concentration and pressure.

E) Filtration – Bubble point pressure test. It checks integrity and pore size of filter.

F) HEPA filter – DOP test. Sodium dioctyl phthalate particles - 0.3 µm size

Retention efficiency of these particles is determined.

References:

1. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

Fungi (Lecture 21)

Fungi are eukaryotic chemoorganotrophic organisms. Molds consists of two parts – mycelium and spores. Mycelium is complex of several filaments called hyphae. The cell walls contain chitin. Some hyphae may divided by cross sections called septa. Hyphae are of three types -

1. Nonseptate or coenocytic
2. Septate with uninucleate cells
3. Septate with multinucleate cells

Importance of fungi

1. Saprophytes – degrade complex organic matter
2. Industrial fermentations
3. Parasites – cause human, animal and plant infections. Human infections caused by fungi –
   - Fungal skin infections – skin, nails and hair
   - Internal organs - Histoplasmosis
5. As a tool for study of physiology, genetics, biochemistry
6. Fungi produce many products used in the medical field such as penicillin, cephalosporin antibotics, cortisone
7. Fungi are used in genetic engineering – vaccine for hepatitis B was developed using the yeast plasmid as the vector.

Cultivation of fungi - Selective media: high sugar conc., acidic pH, can be made containing antibacterial antibiotic.

Ex. Sabouraud's ag, Czapek Dox agar

Optimum temperature – 20 -30^{o} C.

References:

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.
2. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed.,

VIRUS INTRODUCTION – GENERAL CHARACTERS (LECTURE 22)

Viruses are obligate intracellular parasites of human, animals, plants, bacteria.

They are 20-300 nm in size.

Ultramicroscopic (seen only under electron microscope)

They do not have cellular structure.

They are nucleocapsids.

They have only one type of nucleic acid, either DNA or RNA.

Structure of virus

Nucleic acid is enclosed in capsid.

Capsid is made up of protein. (nucleoproteins)

Capsid is made up of morphological subunits called capsomeres.

Nucleic acid of viruses

NA may be single or double stranded.

RNA may be single or double stranded.

DNA may be single or double stranded.

1. Spherical viruses –

It is a regular polyhedron with 20 Triangular faces. 12 vertices and 30 edges. Ex. – poliovirus and adenovirus.

2. Helical symmetry -rod shaped

Its NA is covered by a capsid containing capsomeres arranged in regular helix.

Ex. – Measles, mums, influenza, rabies and TMV.

3. Complex symmetry

Ex. - Poxviruses

They consist of many layers of proteins and lipoproteins. They are brick shaped.

Ex. - Bacteriophages - Binal symmetry

They have icosahedral head attached to helical tail.

Refereces:

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.

2. Anathnarayan R. and Panikar C. K. J., (2000). Textbook of Microbiology, 6/Ed., Orient longman.

CULTIVATION OF VIRUSES (LECTURE 23)

Viruses are obligate intracellular parasites. They can not grow on nutrient media. Viruses can grow only in living cells.

Methods of cultivation:

1. Embryonated chicken eggs
2. Tissue culture
3. Laboratory animals

Tissue culture

Today this is the method of choice.

When viruses grow in tissues, tissue structure deteriorates. It is called as cytopathic effect.

Cell cultures are of 3 types –

a. Primary tissue culture

b. Diploid cell strains

c. Continuous cell line

Bacteriophages do not have their own metabolic machinery.

They can replicate only in bacterial cell. They are host specific.

Refereces:

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.
2. Anathnarayan R. and Panikar C. K. J., (2000). Textbook of Microbiology, 6/Ed., Orient longman.

Multiplication of viruses(lecture 24)

Lytic cycle of bacteriophage

It occurs in following steps

1. Adsorp tion
2. Penetration
3. Transcription of viral proteins
4. Assembly
5. Lysis and release

Lysogeny

Bacteriophage enters into bacterial cell but does not produce lysis of bacteria.

Ex. – lambda phage of *E. coli*

Multiplication of animal viruses

1. Attachment - Viruses attaches to cell membrane
2. Penetration - By endocytosis or fusion
3. Uncoating - By viral or host enzymes
4. Biosynthesis -Production of nucleic acid and proteins
5. Maturation - Nucleic acid and capsid proteins assemble
6. Release - By budding (enveloped viruses) or rupture

Refereces:

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.
2. Anathnarayan R. and Panikar C. K. J., (2000). Textbook of Microbiology, 6/Ed., Orient longman.

FACTORS AFFECTING ACTION OF DISINFECTANT (LECTURE 25)

Disinfection :It is the process of reducing the number of pathogenic microorganisms to the point where they will no longer cause diseases.

1) Concentration of disinfectant

Rate of killing increases with concentration of disinfectant.

2) Time of contact

To kill resistant bacteria disinfectant should be in contact with surface for long time.

3) Temperature

Bactericidal activity of disinfect increases with increase in temperature within limits.

4) pH

pH affects potency of disinfectant and its ability to bind cell surface.

5) Divalent cations

Divalent cations Ca^{++}, Mg^{++} interact with cell surface and block attachment of disinfectant.

6) Extraneous organic matter

Activity of disinfectant decreases in presence of extraneous organic matter.

7) Natural resistance of microorganism

Microorganisms vary tremendously in their susceptibility to disinfectant. Bacterial endospores, mycobacteria, prions are most resistant.

7) Microbial density

Larger the number of microorganisms present, longer it takes a disinfectant to kill cells.

References:

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.

2. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

DISINFECTANT HALOGENS AND PHENOLS
(LECTURE 26)

Halogens: chlorine and iodine are used for disinfection

- **Iodine :**Aqueous solution / Alcoholic solution of iodine and Iodophores are used for disinfection.
  Application of iodine
  Skin antiseptic, Disinfection of water, Sanitization of food utensils
- **Chlorine:** Cl2 gas, Hypochlorites, Chloramines
  Applications of Chlorine
  Disinfection of water, . Household disinfectant.
  Sanitization of utensils in food and dairy industry.

Phenols:

Phenols are powerful microbicidal substances.

Eg: Lysol, cresol, hexylresorcinol.

Mode of action- 1. Disruption of cells 2. Precipitation of proteins 3. Inactivation of enzymes

Applications

1. Disinfection of sputum, urine, feces.
2. Disinfection of utensils, instruments.
3. Milder phenolics – antiseptic
4. Aqueous solutions are used in treatment of wounds

References:

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.

2. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

DISINFECTANT – ALCOHOLS AND SURFACEACTIVE AGENTS
(LECTURE 27)

ALCOHOLS

Ethanol /Isopropyl alcohol are frequently used. No action on spores. Concentration recommended 60-90% in water.

Alcohol	Phenol coefficient
Methanol	0.03
Ethanol	0.039
Isopropyl alcohol	0.054

Mode of action of alcohol

1. Protein denaturation
2. Cell membrane damage
3. Dehydration of cell
4. Inhibition of enzymes

Practical applications

1. Disinfection of clinical thermometer.
2. Antiseptic for the skin – Venupuncture.
3. About 60 % effective against viruses.

SURFACE ACTIVE AGENTS

1. **Anionic detergents** – soap, sodium lauryl sulfate.
2. **Cationic detergents** - Quaternary ammonium compound , cetylpyridinium chloride.

Quaternary ammonium compounds

Most germicidal detergents. Effective against bacteria, fungi and protozoa.

Mode of action: Mechanical removal of microorganisms by decreasing surface tension. Denaturation of proteins. Interference with glycolysis. Damage to cytoplasmic membrane

Applications: Skin disinfectant. Preservative in ophthalmic solutions and cosmetic preparations. Disinfectant - hospital floors and walls. Sanitizer in food industry.

References:

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.
2. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

DISINFECTANT:HEAVY METALS AND COMPOUNDS

(LECTURE28)

Mercury

Inorganic mercury compounds - Mercuric and mercurous chloride, Mercuric oxide. Bactericidal - 1:1000 dilution. Corrosive, toxic to animals. **Applications** – Insoluble compounds used in ointments as antiseptic.

Organic mercury compounds - Mercurochrome, Merthiolate , Metaphen Less irritating and less toxic than inorganic compounds. **Applications** – antiseptic on cutaneous and mucosal surface.

Silver

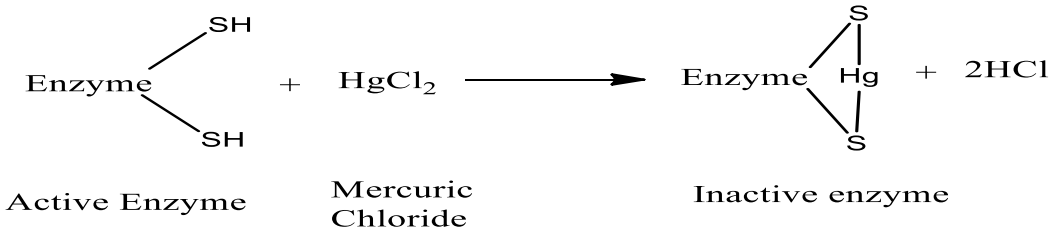
Silver nitrate, Silver lactate, Silver picrate, Silver nitrate – 1:1000 dilutions – bactericidal. **Applications** – Antiseptic treatment of ophthalmia neonatorum.

Copper

Copper sulphate. More effective on algae and fungi than bacteria.

Applications - Prevent algal growth in water – 2 ppm . Component of Bordeaux mixture

Mode of action



References:

1. Pelczar M. j. et al., Microbiology,5/Ed., Mcgrow Hill, New York, 1986.
2. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

EVALUATION OF DISINFECTANT (LECTURE 29)

1. Redeal Walker Test

RW test is used for evaluation of disinfectant. Phenol is used as standard disinfectant. Activity of test disinfectant is compared with phenol and expressed as phenol coefficient. Standard test organism used is *S. typhi*.

Procedure

1. Different dilutions of phenol and test disinfectant are prepared.
2. Dilutions of phenol are in the range of 1:90 to 1: 110
3. Reaction mixture – 5ml of dilution + 0.2ml culture of *S. typhi.*
4. Incubation temperature of reaction mixture at 17 – 18 ^{o} C .
5. Sampling time – 2.5, 5, 7.5, 10 minutes.
6. Samples are added to 5 ml of nutrient medium.
7. Incubation - 37 ^{o} C for 72 hrs.

RW **coefficient =** $\frac{\text{Dilution of disinfectant killing microorganisms in 7.5 not in 5 min}}{\text{Dilution of phenol killing microorganisms in 7 not in 5 min}}$

2. Kelsey and Sykes Test

Kelsey Sykes test is used for evaluation of disinfectants. This test does not require standard disinfectant.This test evaluates effectiveness of disinfectant to deal with successive contamination.The test can evaluates effect of disinfectant in presence of organic matter.

References:

1.Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.
2.Rawlins E. A., (eds.), Bentley's Textbook of Pharmaceutics, 8/Ed., Bailliere Tindall, London, 1992.

STERILITY TEST (LECTURE 30)

It is performed to detect presence of viable microorganisms in sterile pharmaceutical products.

Products tested – Parenterals, Ophthalmic preparations, Dressing, Surgical instruments, Implants, Catguts

Principle - If pharmaceutical product is added to nutrient medium and if it contains microorganism then after incubation they will grow and produce turbidity.

Culture media - I.P.suggests use of joint media

1. Fluid thioglycollate medium or alternate Fluid thioglycollate medium

Supports growth of aerobic and anaerobic bacteria.

2. Soyabean casein digest medium - Supports growth of aerobic bacteria and fungi.

Control test - 1) Test for sterility of medium
2) Growth promotion medium
3) Test for fungistasis and bacteristasis

Methods for sterility test

A) Membrane filtration method

Used for following substances - Oils, Ointments, Substances with antimicrobial activity, Solids not soluble in nutrient medium, Liquids of more than 100ml volume

B) Direct inoculation method

Pharmaceutical product is directly added to nutrient media.

Incubate FTM and SCDM at appropriate conditions for 14 days.

Interpretation -

No growth – sample passes sterility test, Growth – sample fails sterility test

Repeat test – only if Growth in negative control tube or Working area found to non-sterile

References:

Indian Pharmacopia, Govt. of India, Ministry of health and family Welfare. 1996 and 2007.

DESIGNING OF ASEPTIC AREA (LECTURE 31)

Aim - To prevent contamination of microorganisms during preparation and testing of sterile pharmaceutical products.

Definition - Aseptic area is a room with in a clean area designed, Constructed, Serviced and Used with intension to prevent microbial contamination of product.

Factors considered during designing of aseptic area

1. Site -Separate from general manufacturing area.
2. Size - Smallest. No storage facility. No washing and changing facility. No entry to unauthorized person
3.Windows - Purpose – illumination and not ventilation. Large windows of clean glass, Non-operable
4. Doors - Air lock.. Swing doors – fit better

5.Floor walls, bench tops should be easy to clean and smooth, impervious , resistant to chemicals

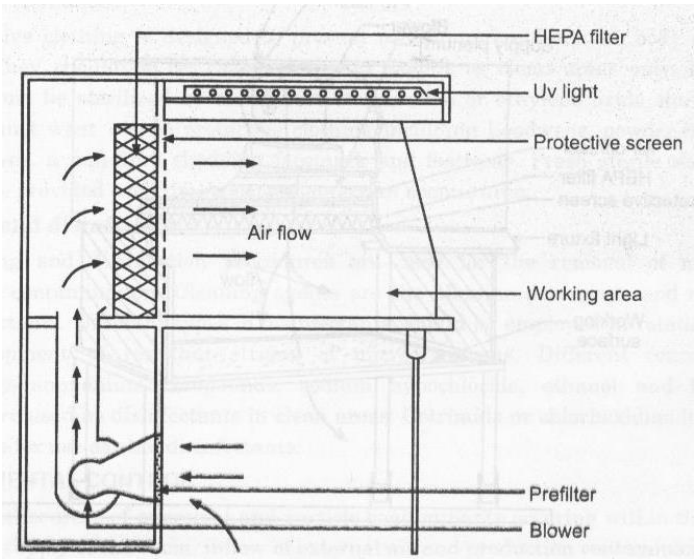
6. Services - a. Ventilation – temperature, humidity and microorganisms of air should be controlled. b. Pipe lines of gas, water and electric wires should be through walls. C. Lights should be fitted in ceiling – to avoid dust and air flow. d. Electricity switches should be outside the aseptic area
e. Vacuum cleaning and not sweeping – dry cleaning. f Wet cleaning – different disinfectants in rotation. g. Air supply Air is filtered through HEPA filter to remove particulate and microbial contamination

References:

1. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.
2. Kokare C.R., Pharmaceutical Microbiology – Principles and Applications, 6/Ed., Nirali Prakashan, pune, India 2008.

LAMINAR AIR FLOW EQUIPMENTS (LECTURE 32)

- Air is filtered through HEPA filter to remove particulate & microbial contamination
- HEPA filter – these are filters are 99.99% efficient in removing particles of size 0.3µm or larger
- Fiber glass + acrylic resins
- Air velocity – 100±20 ft/min
- Temp. – 20-22^{0}C
- Humidity – 35-50%
- Efficiency tetsting - DOP test



Air flow in aseptic area:

It should generate least particle from walls, floor & operator

Types –

- Unidirectional air flow
- Non unidirectional air flow
- Combined air flow

References:

1. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

2. Kokare C.R., Pharmaceutical Microbiology – Principles and Applications, 6/Ed., Nirali Prakashan, pune, India 2008.

SOURCES OF CONTAMINATION IN AN ASEPTIC AREA (LECTURE 33)

1. Atmosphere- Microorganisms from soil, human activities, handling of contaminated material are added to air.These microorganisms remain in air for some time & settle down after some time.
2. Operator: Microorganisms from skin, hair, clothing, nasal passage,
3. Raw material: Natural raw material – no. of organisms is high than synthetic material
4. Water: Used for cleaning, washing, preparation of product. It always contain microorganisms
5. Building: Wall, floor of building contain microorganisms
6. Equipment: a serious source of contamination to pharmaceutical products. Valves, joints, pumps in equipment should be minimum.
7. Working surface
8. Packing: Glass containers, cardboard boxes, closures, liners act as source of contamination.

References:

1. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.
2. Kokare C.R., Pharmaceutical Microbiology – Principles and Applications, 6/Ed., Nirali Prakashan, pune, India 2008.

PREVENTION OF CONTAMINATION TO ASEPTIC AREA

(LECTURE 34)

Following measures are taken to prevent contamination to aseptic area:

- Site of aeptic area slected is away from corridors, stairways & lifts
- Size: Smallest without storage, washing & changing facility
- Access to aseptic area should be through one or more rooms
- Windos: Non-operable only for illumination
- Doors: Air lock system. Double door about 1meter apart. Swing doors – fit better
- Walls and floor: Non-inflammable, fire resistant. Made up of tiles, glass, stainless steel. Cupboards, drawers, shelves & equipments in roomservices: should minimum
- Pipe lines of gas, water & electric wires should be through walls. Lights should be fitted in ceiling – to avoid dust & air flow. Electricity switches should be outside the aseptic area Person: Number of persons working area should be minimum. Arrangements in room should be such that there will be minimum movements of working persons

References:

- 1. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.
- 2. Kokare C.R., Pharmaceutical Microbiology – Principles and Applications, 6/Ed., Nirali Prakashan, pune, India 2008.

CLASSES OF CLEAN AREAS (LECTURE 35)

Clean rooms are classified according to number and size of particles permitted per volume of air.There are different systems of classification of clean rooms

US FED STANDARD

Class	Property	Use
Class 100 clean room	Particle count not more than 100/cu. Feet of 0.5µm or larger. Expensive, high grade cleanliness, difficult to maintain	Perfect sterile working area for parenterals.
Class 10,000 clean room	Particle count not more than 10,ooo/cu. Feet of 0.5µm or larger.	Buffer area around class 100 room. Handling of precleaned containers, aseptic gowning, process filtration
Class 100,000 clean room	Particle count not more than 100,000/cu. Feet of 0.5µm or larger	Finish packing, stock staging, laboratory

EU GMP CLASSIFICATION:

Grade	Viable $m.o./m^3$ of air	Settle plate count-9cm/4 hrs.	Contact plate 5.5cm	Glove print five finger
A	Less than 1	Less than 1	Less than 1	Less than 1
B	10	5	5	5
C	100	50	25	NA
D	200	100	50	NA

References:

1. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.
2. Kokare C.R., Pharmaceutical Microbiology – Principles and Applications, 6/Ed., Nirali Prakashan, pune, India 2008.

MICROBIAL ASSAYS (LECTURE 36)

Micrbiological assays include both assays in which compound to be quantitated either depresses or stimulates growth of sensitive test microorganism.

Antibiotics, vitamins, amino acids, growth factors

Advantages: Can be performed even if compound to be assayed is present in complex mixtures. Detects only biologically active compound.

Disadvantages: Produce greater error, Less reproducible Produce greater error, Less reproducible, time consuming.

Methods of assay

1. Diffusion assays: Use solid medium. Compound to be assayed diffuses through medium in radial fashion from cup or disc. So that adjacent growth of the test organism is either depressed by antibiotic or stimulated by vitamin.
2. Turbidimetric assays: Use liquid medium and measure the growth of microorganism by measuring turbidity
3. End point determination assay

Assessment of a new antibiotic and testing of antimicrobial activity of a new substance. (Lecture 37)

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance.

Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods.

Several bioassays such as disk-diffusion, well diffusion and broth or agar dilution are well known and commonly used.

Flow cytofluorometric and bioluminescent methods are not widely used because they require specified equipment, even if they can provide rapid results of the antimicrobial agent's effects and a better understanding of their impact on the viability and cell damage inflicted to the tested microorganism.

Different methods of antimicrobial testing:

1. Diffusion method- Agar well, Agar Disk ,Agar Plug
2. Broth dilution method
3. Agar dilution
4. Time kill test

General aspects-environmental cleanliness. Lecture 38

Environmental cleaning**:** processes associated with cleaning and disinfection of surfaces and substances that can pose a risk of harm to patients

Contaminated hospital surfaces play an important role in the transmission of dangerous pathogens, including *Clostridium difficile*, and antibiotic-resistant organisms such as methicillin-resistant *Staphylococcus aureus*(MRSA) and vancomycin-resistant enterococci (VRE).

Therefore, appropriate disinfection of those surfaces and equipment which patients and healthcare personnel touch is necessary to reduce exposure.

Disinfection Strategies

There are a wide variety of chemical disinfectants approved for use in the hospital setting.

The most commonly used surface disinfectants are quaternary ammonium compounds and sodium hypochlorite.

The effectiveness of chemical disinfectants can depend:

1. The antimicrobial activity of the disinfectant and
2. Adequacy of cleaning,
3. Appropriate contact time, and
4. Concentration of the disinfectant.

Terminal disinfection of empty rooms can be done by ultraviolet light or fogging with hydrogen peroxide vapor or mist.

Types of spoilage of pharmaceutical products (lecture 39)

Definition: Spoilage of pharmaceutical product is presence of low number of pathogens or toxic microbial metabolites of microorganisms and detectable physical or chemical changes occurring in pharmaceutical product.

Types of spoilage

- Infections caused by contaminated pharmaceutical products.
- Physical and chemical deterioration of product.
- Observable effects of microbial attack on product.
- Ingredients susceptible to microbial attack

Contaminating organisms and their infections

Dosage form	Contaminating m.o.	Infections
Tablet or capsule	*Salmonella spp.*	Salmonella infection
Eye drops	*P. aeruginosa*	Eye infection
Antiseptic solution	*Pseudomonas spp.*	Septicemia
Ointments and creams	Gram negative bacteria	Dermatoses and burns
Intravenous medicines	*Candida spp.*	Fatal septicemia

References:

1. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

Factors affecting the microbial spoilage of pharmaceutical products (Lecture 40)

Physical and chemical status of pharmaceutical formulation influence the type and extent of microbial spoilage.

1. Size of inoculums
2. Nutritional factors**:** Complex pharmaceutical products contain variety of nutrients.- Crude animal and vegetable products. This supports growth of appreciable microorganisms which results into spoilage of product.
3. Moisture content: Higher level of water content supports good growth of bacteria. A_w is decreased by addition of sugars, salts, or polyethylene glycol
4. Temperature: Spoilage of pharmaceutical product could occur over temperature range -10 to 60^0C. Spoilage is less at extremes of temperature range
5. pH: Extremes of pH prevent microbial attack. More spoilage at neutral pH.
6. Redox potential: more groth of organism in presence of air/ oxygen

References:

1. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

Sources and types of microbial contaminants of pharmaceutical products (Lecture 41)

1. Atmosphere- Microorganisms from soil, human activities, handling of contaminated material are added to air.These microorganisms remain in air for some time & settle down after some time.
2. Operator: Microorganisms from skin, hair, clothing, nasal passage,
3. Raw material: Natural raw material – no. of organisms is high than synthetic material
4. Water: Used for cleaning, washing, preparation of product. It always contain microorganisms
5. Building: Wall, floor of building contain microorganisms
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2. Kokare C.R., Pharmaceutical Microbiology – Principles and Applications, 6/Ed., Nirali Prakashan, pune, India 2008.

Assessment of microbial contamination and spoilage.

(Lecture 42)

- Physical and chemical change
- Sterility test
- Assessment of viable microorganisms in non-sterile products
- Estimation of pyrogen

Microbial limit tests

- To assess microbial quality of: **non-sterile** pharmaceutical products - Non-sterile **finished products** – calamine lotion, dried aluminums hydroxide gel
- **Raw materials** of natural or biological origin - e.g. starch, gum, gelatin, talc.

It involves two types of tests -

A. To determine total aerobic viable count: Bacteria and fungi

- Membrane filtration method
- Total plate count
- Most probable number by multiple tube method

B. To detect presence of specific microorganism

- E.coli
- Salmonella
- Pseudomonas aeruginosa
- Staphylococcus aureus
- Candida albicans

References:

1. Hugo W. B. and Russell A. D. Pharmaceutical Microbiology, 6/Ed., Blackwell Science, 1988.

2. Kokare C.R., Pharmaceutical Microbiology – Principles and Applications, 6/Ed., Nirali Prakashan, pune, India 2008.

Preservation of pharmaceutical products using antimicrobial agents (Lecture 43)

A chemical substance that is used to preserve pharmaceuticals, from decomposition or fermentation by preventing the growth of microorganisms often termed as Antimicrobial Preservatives.

Preservatives are substances that are commonly added to various foods and pharmaceutical products in order to prolong their shelf life.

The addition of preservatives to such products, especially to those that have higher water content, is essential for avoiding alteration and degradation by microorganisms during storage.

Ideal Properties of Preservatives

1. It should not be irritant and toxic.
2. It should be physically and chemically stable and compatible with other ingredients used in formulation.
3. It should exert wide spectrum of activity.
4. It should act as preservative in small concentration i.e. it must be potent.

Examples of Antimicrobial preservatives include:

1. Methyl, ethyl, propyl and butyl Parabens: Parabens are among the most commonly used preservatives. They are relatively active against a broad spectrum of microorga. Parabens are more effective against gram negative than gram positive organisms.

2. Sorbic acid. Na, K & Ca Sorbate,

3. Benzoic acid, Na, K & Ca Benzoate,

4. Sodium metabisulfite,

5. Propylene glycol (15-30%).

6. BHT, BHA, benzaldehyde,

7. Phenol and Mercury compounds.

Animal cells culture (Lecture 44)

Cell culture: It is a process through which cells are isolated from animal and plant and then grown under controlled artificial conditions.

Types of cell culture

1. Primary cell culture: It is the growth of the cells dissociated from parental tissue (kidney / liver) using mechanical or enzymatic methods in culture medium using glass or plastic con

2. Secondary cell culture: When primary cell culture is sub-cultured , it **becomes secondary cell culture or cell line**

3. Established cell lines: Established cell lines are of two types depending on life span of culture

- Finite cell lines - has limited culture life span
- Continuous cell lines – immortal in culture

4. **Transformed cell culture**: Sometimes eukaryotic cells exhibit unusual and alternative growth characteristics both in culture and in vivo.

- No contact inhibition
- Not anchorage dependant – permitting to grow in multilayers
- Grow to indefinite population size
- Growth continues for many generations
- Cells grow irregularly in size and shape and often reveal abnormal chromosome content

Application of cell cultures (Lecture 45)

1. Model system – Cell culture is used to study basic cell biology and biochemistry, interaction between cell and pathogens, effect of drugs, process of aging.
2. Cancer research – To understand mechanism and cause of cancer. To find out drugs that selectively destroy only cancer cells.
3. Virology – For cultivation of viruses, viral vaccine preparation.
4. Toxicity testing – to study effect of new drugs, cosmetics, chemicals on different types of cells like liver, kidney cells. To determine maximum permissible dosage of new drug.
5. Genetically engineered protein – To produced genetically engineered proteins as insulin, monoclonal Abs, hormones etc
6. Production of tissues and organs for replacement - Artificial skin can be produced to treat patients with ulcers and burns. Research is going on the production of artificial organs like liver, kidney pancreas.
7. Genetic counseling – fetal cells from pregnant can be used to check chromosomal abnormalities.
8. Genetic engineering – new genes can be introduced into cultured cells..
9. Gene therapy –
10. Cells from the patient lacking functional gene are isolated.
11. Functional genes can be introduced into cells.
12. Such cells are reintroduced into a person can be used fro treatment of genetic disorders.
13. Drug screening and development – Animal cell culture an be used to study:
    a. Cytotoxicty of drug
    b. Effective and safe dosage for new drug