Program	:	B. Pharmacy
Name of Unit	:	Spoilage, microbial stability and cell culture and its applications.
Subject /Course name	:	Pharmaceutical Microbiology
Subject/Course ID	:	BP 403T
Class: B.Pharm. Semester	:	III
Module	:	V
Course coordinator	:	Sharanpreet Kaur
Mobile No.	:	9465591459
Email id	:	sharankashyap1@gmail.com

Learning Outcome of Unit-5

LO	Learning Outcome (LO)	Course Outcome Code
LO1	Students will learn about antimicrobial agents	BP303.3,4
LO2	Students will learn about cell culture and its application in pharmaceutical industry.	BP303.4

Module Content Table

No.	Topic
1	Types of Spoilage, factors affecting the microbial spoilage of pharmaceutical products, sources and types of microbial contaminants, assessment of microbial contamination and spoilage.
2	Prevention of pharmaceutical products using antimicrobial agents, evaluation of microbial stability of formulations.
3	Growth of animal cell culture, general procedure for cell culture, primary establishment and transformed cell cultures.
4	Application of cell cultures in pharmaceutical industry and research.

SPOILAGE:

Spoilage is chemical and physicochemical degradation of pharmaceutical products rendering it unsuitable for use.

Spoilage is not desirable in pharmaceutical industry because deterioration of drugs and excipient occurs, as a result product may lose its quality and it may become ineffective. Entire batch may be required to be discarded. This may cost huge lose to the manufacturer. Moreover it may attract litigation from the consumers which may cause huge financial loss to the Company.

Damaged products may damage reputation of the manufacturing company which may attract further financial loss. Microbial spoilage may present potential health hazards to the consumers like toxicity, infection or even death. Toxic metabolites may be produced due to microbial growth which may cause health hazards. Microbes may deteriorate drugs and thereby reduce potency of the medicament. There could be change in the appearance of the product like discoloration, phase separation, and odor formation etc. Following types of microbial spoilage occurs in pharmaceutical products.

1. Chemical spoilage.
2. Physicochemical spoilage
3. Biological spoilage.

Chemical Spoilage - Chemical spoilage means deterioration of chemical nature of drugs and excipients. Molecular structure of the ingredients may change. This change may affect physicochemical properties of the preparation. Potency of the drug may decrease. Further microbial growth may occur if chemical degradation of preservative occurs.

Similarly chemical degradation of surfactants, organic polymers etc may damage specialized micro-environment for which they are used. Along with microbial contamination some - chemicals may also cause chemical spoilage like pesticide, disinfectants, bleaching agent, sanitizer etc. may add to chemical spoilage.

Physicochemical spoilage - This type of spoilage may change physico-chemical properties of product. Following types of changes may occur.

I. Viable growth - Viable growth of microbes may occur inside the container. These growth may be visible in the form of floating layers, turbidity, hemps, etc. Contamination of products by fungus species like *Aspergillus sp.* may cause this type spoilage. Some bacterial species may also show viable growth.

II. Gas production -Some microbes may produce gas inside the containers. These gases may be visible in the form of bubbles, floccules etc. Contamination of products with bacteria like *E. coli* may produce gas if it contains sugars.

III. Coloration / Decoloration -Some microbes may decolorize formulation or it may produce unique color which is different from the normal colour of the product.

IV. Odor formation-Microbial growth in the finished product may produce bad odour or characteristic odor. It may produce a characteristic rotten smell.

V. Taste change -Microbial spoilage may change the taste of the oral formulations. It may impart bitter or obnoxious taste to the oral formulation.

Biological Spoilage - Spoilage of pharmaceuticals may produce some undesirable and dangerous molecule which has undesirable biological effects. Some microbes may produce toxins, pyrogens or other harmful metabolites. These biomolecules may be present in the product from the very beginning. Spoilage may occur, although no microbial contamination was there.

Factors affecting the microbial Spoilage of the Pharmaceutical products

There are so many factors which affects Microbial spoilage. Some of these factors reduce rate of spoilage where as some factors increases the rate of spoilage. These factors are related to nutritional requirement of micro-organisms, environment and nature of micro-organism. These factors must be studied to minimize the impact of spoilage. Following factors affect the microbial spoilage of Pharmaceutical products.

Number of contaminating micro-organism - There may not be sufficient spoilage if number of contaminants is less. This low level it contaminants may not able to multiply due to design of the formulation. However during long storage these microbes may cause damage. If initial contamination is high then it may present an undesirable challenge. Products will be damaged quickly due to presence of high numbers of microbes. High level of contamination occurs due to lapses in manufacturing process or due to high loads of contaminants in raw materials.

Type of micro-organism -Some microorganisms is more aggressive than others. They can quickly multiply and spoilage is much faster. However, aggressive microbes may not multiply initially due to presence of preservative and other substances. Contamination of product with natural communities of non-aggressive micro-organism can facilitate growth of other aggressive contaminants.

Presence of nutrients - In product Micro-organism can utilize formulation components as nutrients and utilize these components for biosynthesis and growth. Many formulations may contain crude animal, vegetable and microbial products which creates a conducive environment for microbial growth and subsequent spoilage. Additives like sugar, amino acid, polyhydric alcohol may act as microbial nutrients. Primary contaminants may produce metabolites which could be used by aggressive microbes as nutrient. Demineralized water which is prepare by ion-exchange resins may contain nutrients.

Presence of water - Presence of water in formulation may promote microbial growth and subsequent spoilage. Uncomplexed water or free excess water promotes this spoilage. Presence of free water may be measured by water activity. It is a ratio between 'vapor pressure of formulation and vapor pressure of water in similar condition'. If water activity is 1 then it is conducive for microbial growth. Chances of microbial growth decreases with decrease in value of water activity. A value less than 0.88 is considered safe to prevent spoilage. However, some microbes can grow in extremely low water value condition. - *Aspergillus glaucus* can grow at a water activity value of 0.61.

Oxidation-reduction potential- Contaminating micro-organism may requires terminal electron accepter to facilitate functioning respiratory pathway. Presence of dissolved oxygen increases redox potential of the product thereby promote microbial growth and spoilage.

Temperature -Storage temperature is a great controller of microbial growth and spoilage. However, spoilage may occur over a range -20^{o}C to 60^{o}C. Microbial growth and spoilage is less in low temperature and high temperature. Storing products at a cool place (8^{o}C to 12^{o}C) may cause negligible spoilage. High temperature can also prevent spoilage. Water for injection is stored above 80^{o}C before filling and sealing. So extreme temperature can minimize spoilage.

pH- Microbes prefer neutral pH for their growth. However some microbes may prefer slightly acidic pH. Extremely acidic or alkaline pH of the formulation may prevent microbial growth and subsequent spoilage. Some communities of microbes can survive in extreme pH and change the pH, there by support growth of other micro-organism.

Containers and packaging - Chances of contamination in single dose ampoules and vials are negligible. However multi dose containers be contaminated by users themselves. Change in designs of these containers may minimize contamination and spoilage. Wide mouthed containers for creams and ointments are replaced by narrow mouthed tubes with screw cap closures. Multi dose injections are stored in containers which has self sealing rubber wads. Containers are sealed such a way so that it can prevent entry of water and oxygen to minimize water activity and redox potential.

Presence of protection materials - Some materials in formulation may protect microbes during sterilization process. Polymers like gelatin, starch ete may increase microbial resistance to heat. Microbes may get adsorbed in particles and become more resistant to heat.

Source and types of microbial contaminants-

Microbes are very important part of our environment. They are present almost anywhere and everywhere. So, contamination may occur to pharmaceutical products in large scale manufacturing, in small scale hospital manufacturing or during use by the patient. Following are

the different source of microbial contamination in pharmaceutical products.

In large scale manufacturing: - In large scale manufacturing as well as medium and small scale manufacturing contamination may occur from following sources.

Water -Water is a major source of contamination. Common water borne micro-organism like Pseudomonas, Achromo bacteria and other low demand gram negative groups are present in portable water as well as in purified water. Ion-exchange column may be contaminated by water source and micro-organism may multiply there to contaminate purified water.

Water which is purified by reverse osmosis process may also be contaminated if osmosis membrane is no properly disinfected. Even distilled water may also be contaminated if it is stored for few day at normal temperature.

Raw materials - Pharmaceutical products are prepared from varieties of raw materials. Clays and earth materials like bentonite, kaolin ete may contain anaerobia spores like *Clostridium sp*. Starch may contain coliform batteria like *E. Coli.* Gums may contain actinomycetes. Animal Products may contain a variety of bacterial like *E. coli, Salmonella sp* etc.

Air of the manufacturing area:

Air is filled with billions of suspending particles and microbes. Fungus spores, like penicillium, mucor, aspergillus ete. Bacterial spores like *Bacillius sp*. etc are also present. These spores and micro-organism may contaminate pharmaceutical products. This type of contamination is minimized by practice of manufacturing in clean room and in aseptic room under continuous flow of sterile air through HEPA filter.

Personnel- manufacturing staff may also contaminate pharmaceutical products. Personnel may be infected with various types of infections like coliform bacteria, staphylococci, strepto cocci, Actino bacteria, Candida. This types of contamination may be minimized by pooper health check-up, vaccination and hygiene of the personnel. Protective gear and pooper training of the personnel may also minimize the contamination.

Equipments -

Equipments of manufacturing may contain microbes if it is not sterilized properly. Grinder, blender, filter etc may contain non-specific and local communities of micro-organism.

Containers: containers may cause contamination if it is not sterile.

In hospital manufacturing -In hospital manufacturing water and environment are the major source of contaminants. In hospitals, water is stored in storage tank which may develop fungus, bacteria and algae type of microbes. Hospital air may be contaminated with pathogenic micro-organism due to the presences of infected patients and numerous visitors.

Preservation of pharmaceutical products using antimicrobial agents:

Antimicrobial agents are those substances which can kill of inhibit growth of micro-organism. These antimicrobial agents are included in formulation in order to minimize levels of contaminated micro-organism. These antimicrobial agents are called preservatives. These preservatives can generally prevent or kill low levels of contamination. Preservatives are not used in those formulations which has low risk of contamination and subsequent microbial growth. Formulations which contain high level of acid, alkali, sugar etc may not require preservative. Formulations of antibiotics and other anti-microbial substances may not require preservative.

A good preservative should have following characteristics. It should be a broad spectrum antimicrobial agent, *i.e.* it should be effective against variety of microorganisms. However, most active preservatives are ineffective against some microbes.

A good preservative should have a fast killing and inhibition rate and it should selectively react with contaminants and it should not react with ingredients of the formulation. However, most preservatives interact with ingredients of the formulations. A good preservative should be non-irritant and non-toxic to the patients. However, many preservative have toxic effects and cause irritation of skin when used in formulation for topical application. Some preservatives even cause contact dermatitis. A good preservative should be stable and it should remain active throughout the shelf-life of the product. However effectiveness of the preservative may change depending upon so many factors.

Concentration of preservatives determine efficacy of its preservation power. Antimicrobial activity of preservative increases with the concentration. Activity of phenol decreases 64 times if concentration is halved.

Antimicrobial activity of preservative is also dependent on storage temperature. Decrease in storage temperature may reduce killing activity of preservative. It may be calculated by determining temperature co-efficient.

Preservative which is used in formulation may not be available for antimicrobial activity. An unstable equilibrium is formed between preservative and available preservative which controls mass of micro-organism. Unavailable preservative helps to maintain equilibrium by constantly supplying available molecule.

Availability of preservative may also decrease with increased solute concentration and decrease water activity. Ionic and weakly acidic preservatives exhibit their antimicrobial activity when they are not ionized. They produce maximum activity when ionization is very low.

Preservative may distribute itself depending its affinity in a multiphase system. It may distribute itself in water phase and oil phase by an unstable equilibrium. Parameters like partition

coefficient, polymer binding constant and oil-water ratio must be considered while using a preservative in a formulation.

Packaging material can interfere with preservation activity of preservative. Preservative may leach, permeate and interact with rubber, plastic, cork etc.

Different types of preservatives are used in pharmaceutical products. Acid, alkali and esters are used as preservative. Benzoic acid is used as preservative. Its sodium salt, sodium benzoate is also used as preservative. Its ester methyl hydroxybenzoate is also used as preservative.

Some alcohols like chlorobutol, benzyl alcohol etc are used as preservative. Some phenolic compounds like phenol, chlorocresol etc are also used as preservatives.

Some mercuric compounds, such as thiomersal etc are used as preservative. Many other compounds like benzalkonium chloride, cetrimide are used as preservative. Even compounds like chloroform and formaldehyde can be used as preservative.

Assessment of microbial contamination and Spoilage:

Assessment of microbial content of a pharmaceutical product is very essential. Sterile product should be perfectly free from micro-organism and that can be assessed by a test of sterility. However, non-sterile products may contain some micro-organisms. These micro-organisms could be pathogenic and non-pathogenic. All these micro-organisms can cause spoilage and may cause potential health hazards. Number of total micro-organisms present in a products must be low and should be within permissible limit. Types and nature of the microbes should also be assessed to determine the presence of specific microbes.

Total number of microbes and types of microbes is assessed by microbial limit test. Following tests are performed to assess the microbial contamination and subsequent spoilage.

1. Test of sterility:

Indian pharmacopoeia, British pharmacopoeia and United States pharmacopoeia recommends test of sterility for some pharmaceutical products. Procedure are similar with little variations. Indian pharmacopoeia recommended tests are discussed briefly. Two methods are there i.e. direct innoculation and membrane filtration.

1. **Direct inoculation**- In this method, little amount of sample is directly added to the culture media which was specified in pharmacopeia. This inoculated media is then incubated for specified period of time. Presence of growth indicates the presence of micro organism which is sourced from sample. Hence it may be concluded that sample is not sterile. Sample may be termed sterile in absence of any growth.
2. **Membrane filtration**- In this method sample is filtered through a membrane filter and washed with diluting fluid. Microbes, if present, will be there at the top of the filter paper.

Now this filter paper is inoculated into specified culture media. If growth is observed then it indicates that product is not sterile.

A positive and negative control test must also be conducted in both the above mentioned method.

2. Microbial limit test-

The European pharmacopoeia recommends both qualitative and quantitative assessment of micro-organism. United States Pharmacopoeia recommends microbiological Limit test. It has two parts:

(i) Total Aerobic microbial count and

(ii) Test for specified microorganism.

(i) Total Aerobic microbial count-

In this method specified amount of test sample (10gm) is taken and mixed with specified amount (90ml) of peptone water.

This is sample dilution. There are specific procedure to make dilutions for water insoluble products and fatty products. Total microbial count is examined by following procedure-

First, examination of sample by membrane filtration method- 10 ml of prepared dilution is mixed with 90 ml of peptone water and filtered through membrane filter. It is then washed 3 times with sterile peptone water. One filter paper is placed in petri dish containing Soyabean Caesin Digest Agar media and incubated for 5 days at 30-35^{o}C. Bacterial count is determined by counting colonies.

Another filter paper is placed in Sabouraud Dextrose Agar Media and incubated for 5 days at 20-25^{o}C. Fungal count is then determined.

Second, Examination of sample plate count method- In this method prepared dilution is directly transferred to 4 petri dishes. Two for bacteria and two for fungus. In first two petri dishes 15 ml of Soyabean Caesin Digest Agar media is added. Colonies are counter after incubation for 5 days at 30-35^{o}C. In remaining two petri dishes 15 ml of Sabouraud Dextrose Agar Media was transferred and incubated at 20-25^{o}C. Colonies are counted.

(ii) Test for specified micro-organism-

Specific identification tests for *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginea* and *Staphylococcus aureus* is performed.

E. coli is cultivated in Mac Conkes agar media. Colonies are identified by characteristics metalic shine. *Salmonella* are cultivated on bismuth sulphite agar media and identified by black or green colonies.

Pseudomonas aeruginosa is identified by cultivating in cetrimide agar media. Colonies slow

greenish colour.

Staphylococcus aureus is cultivated in Vogel Johnson agar media and identified by black colonies surround by yellow zones.

Periodic test- Periodic test of the product for total microbial count must be done in order to determine continuing efficacy of the product.

Many alternative tests may be done for detection and determination of micro-organism *e.g* Luciferase test, Epifluorescence, electrical impedance etc.

Evaluation of microbial stability of formulation

Microbial stability of a formulation is dependent on effectiveness of its preservative. Chemical assay and biological assay may assure effectiveness of preservative but it may lose its activity due to presence of other ingredients in the formulation. Some formulations do not require preservative because they act as self-preservative. Some formulation does not require preservative because it contains antimicrobial agents like antibiotics as ingredient. Some formulation may contain high sugar concentration, salt concentration and may act as self preservative. So, the ability of the formulation to protect itself from microbial growth must ascertain to determine microbial stability of the formulation. It is done by preservative efficacy test.

Basic principle of this test is to inoculate products with different types of specified micro-organism with specific quantity. Little amount of inoculated product is removed at a specific interval. Then viable count of this withdrawn sample is determined. United States Pharmacopoeia, European Pharmacopoeia etc recommends this type of tests.

The concentration of the test organism should be 10^5-10^6 cells per ml or gm. Total microbial count is performed in 0 hr, 6 hrs, 24 hrs, 48 hrs. 7 days, 14 days and 28 days. British pharmacopeia recommends test even after 28 days.

Different bacterial species are used for this purpose. They are *Staphylococcus aureus*, *Pseudomonus aeruginosa* and *E.coli*.

Different fungus species are also used. They are *Candida albicans, Aspergillus niger* etc. This test allows to add designated micro-organisms if required.

After withdrawal of sample and before viable count, sample is mixed with chemicals which can deactivate preservative because presence of even very minute quantity of preservative may hamper microbial viable count.

There are two types of performance criteria. Criteria A is desired and recommended where as criteria B is satisfactory in justified cases.

Growth of animal cells in culture

Cell culture refers to the process by which cells are grown in a controlled artificial environment. Cells can be maintained in vitro (outside of their original body) by this process which is quite simple compared to organ and tissue culture.

In a cell culture technique, cells are removed from an animal or a plant and grown subsequently in a favorable environment. For animal cell culture the cells are taken from the organ of an experimental animal. These cells may be removed directly or by mechanical or enzymatic action. The cells can also be obtained by previously made cell lines or cell strains. Examples of cells used to culture are fibroblast, lymphocytes, cells from cardiac and skeletal tissues, cells from liver, breast, skin, and kidney, and different types of tumor cells.

Types of animal cell culture

Based on the number of cell divisions, cell culture can be classified as primary cell culture and cell lines. Cell lines can undergo finite or infinite cell divisions.

Animal cell culture

A. Primary cell culture

This is the cell culture obtained straight from the cells of the host tissue. The cells dissociated from the parental tissue are grown on a suitable container and the culture thus obtained is called primary cell culture. Such culture comprises mostly heterogeneous cells and most of the cells divide only for a limited time. However, these cells are much similar to their parents.

Adherent cells

These cells are anchorage-dependent and propagate as a monolayer. These cells need to be attached to a solid or semi-solid substrate for proliferation. These adhere to the culture vessel with the use of an extracellular matrix which is generally derived from tissues of organs that are immobile and embedded in a network of connective tissue. Fibroblasts and epithelial cells are of such types.

When the bottom of the culture vessel is covered with a continuous layer of cells, usually one cell in thickness, these are known as monolayer cultures.

The majority of continuous cell lines grow as mono layers. As single layers, such cells can be transferred directly to a cover slip to examine under a microscope.

Suspension cells

Suspension cells do not attach to the surface of the culture vessels. These cells are also called anchorage- independent or non-adherent cells which can be grown floating in the culture medium. Hematopoietic stem cells (derived from blood, spleen, and bone marrow) and tumor cells can be grown in suspension. These cells grow much faster, do not require the frequent

replacement of the medium, and can be easily maintained. These are of homogeneous types and enzyme treatment is not required for the dissociation of cells; similarly, these cultures have a short lag period.

Confluent culture and the necessity of sub-culture

After the cells are isolated from the tissue and proliferated under the appropriate conditions, they occupy all of the available substrates i.e. reach confluence. For a few days, it can become too crowded for their container and this can be detrimental to their growth, generally leading to cell death if left for a long time. The cells thus have to be subculture i.e. a portion of cells is transferred to a new vessel with a fresh growth medium which provides more space and nutrients for the continual growth of cells. Hence subculture keeps cells healthy and in a growing state.

A passage number refers specifically to how many times a cell line has been sub-cultured. In contrast with the population doubling level in that the specific number of cells involved is not relevant. It simply gives a general indication of how old the cells may be for various assays.

B. Secondary cell culture and cell line

When a primary culture is sub-cultured, it is known as secondary culture or cell line, or sub-clone. The process involves removing the growth media and disassociating the adhered cells (usually enzymatically).

Sub-culturing of primary cells into different divisions leads to the generation of cell lines. During the passage, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population. However, as they are sub-cultured serially, they become different from the original cell.

On the basis of the life span of culture, the cell lines are categorized into two types:

Finite cell lines

The cell lines which go through a limited number of cell divisions having a limited life span are known as finite cell lines. The cells passage several times and then lose their ability to proliferate, which is a genetically determined event known as senescence. Cell lines derived from primary cultures of normal cells are finite cell lines.

Continuous cell lines

When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a continuous cell line. Such transformation or mutation can occur spontaneously or can be chemically or virally induced or from the establishment of cell cultures from malignant tissue. Cell cultures prepared in this way can be sub-cultured and grown indefinitely as permanent cell lines and are immortal.

These cells are less adherent, fast-growing, less fastidious in their nutritional requirements, able to grow up to higher cell density, and different in phenotypes from the original tissue. Such cells grow more in suspension. They also have a tendency to grow on top of each other in multi layers on culture-vessel surfaces.

Common cell lines

Human cell lines:

1. MCF-7 (breast cancer)
2. HL 60 (leukemia)
3. HeLa (human cervical cancer cells)

Primates cell lines: Vero (African green monkey kidney epithelial cells)

Cell strain

Lineage of cells originating from the primary culture is called strain. These are either derived from a primary culture or a cell line by the positive selection or cloning of cells having specific properties or characteristics. A cell strain often acquires additional genetic changes subsequent to the initiation of the parent line.

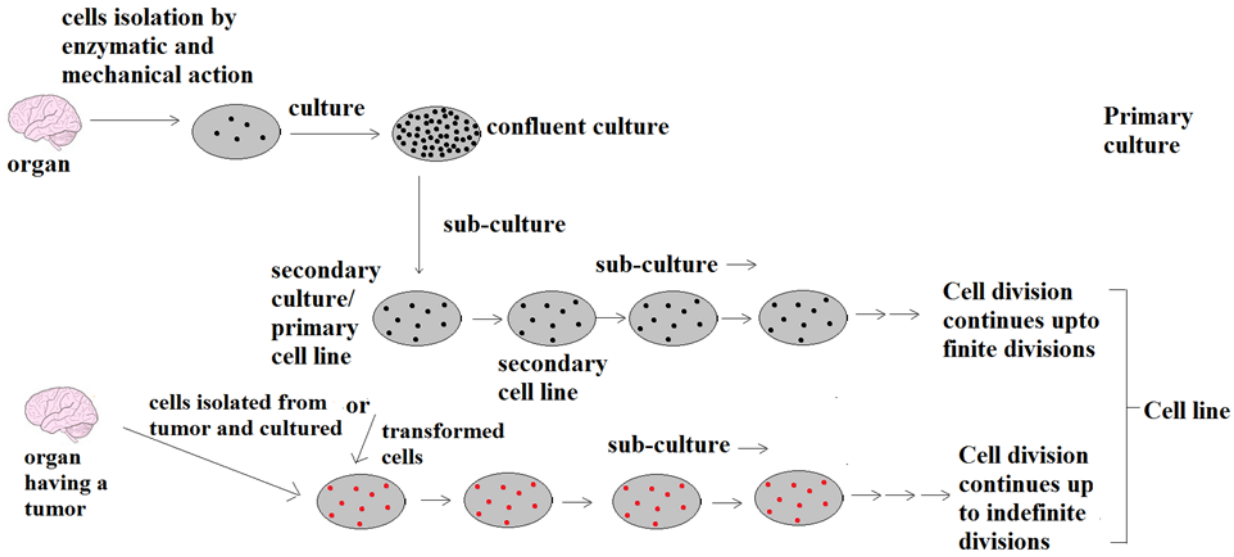

fig: animal cell culture

Methods

Growth Requirements:

The culture media used for cell cultures are generally quite complex, and culture condition widely varies for each cell type. However, media generally include amino acids, vitamins, salts (maintain osmotic pressure), glucose, a bicarbonate buffer system (maintains a pH between 7.2

and 7.4), growth factors, hormones, O_2 and CO_2. To obtain the best growth, addition of a small amount of blood serum is usually necessary, and several antibiotics, like penicillin and streptomycin, are added to prevent bacterial contamination.

Temperature varies on the type of host cell. Most mammalian cells are maintained at 37^0C for optimal growth, while cells derived from cold-blooded animals tolerate a wider temperature range (i.e. 15^0C to 26^0C). Actively growing cells of log phage should be used which divide rapidly during culture.

Process to obtain primary cell culture:

Primary cell cultures are prepared from fresh tissues. Pieces of tissues from the organ are removed aseptically; which are usually minced with a sharp sterile razor and dissociated by proteolytic enzymes (such as trypsin) that break apart the intercellular cement. The obtained cell suspension is then washed with a physiological buffer (to remove the proteolytic enzymes used). The cell suspension is spread out on the bottom of a flat surface, such as a bottle or a Petri dish. This thin layer of cells adhering to the glass or plastic dish is overlaid with a suitable culture medium and is incubated at a suitable temperature.

Aseptic techniques

Bacterial infections, like Mycoplasma and fungal infections, commonly occur in cell culture creating a problem to identify and eliminate. Thus, all cell culture work is done in a sterile environment with proper aseptic techniques. Work should be done in laminar flow with the constant unidirectional flow of HEPA filtered air over the work area. All the materials, solutions, and the whole atmosphere should be contamination-free.

Cryopreservation

If a surplus of cells is available from sub-culturing, they should be treated with the appropriate protective agent (e.g., DMSO or glycerol) and stored at temperatures below –130°C until they are needed. This stores cell stocks and prevent the original cell from being lost due to unexpected equipment failure or biological contaminations. It also prevents finite cells from reaching senescence and minimizes the risks of changes in long-term cultures.

When thawing the cells, the frozen tube of cells is warmed quickly in warm water, rinsed with medium and serum, and then added into culture containers once suspended in the appropriate media.

Applications of Cell Line

A. Vaccines Production: One of the most important uses of cell culture is in the research and production of vaccines. The ability to grow large amounts of virus in cell culture eventually led to the creation of the polio vaccine, and cells are still used today on a large scale to produce

vaccines for many other diseases, like rabies, chickenpox, hepatitis B, and measles. In early times, researchers had to use live animals to grow poliovirus, but due to the development of cell culture techniques, they were able to achieve much greater control over virus production and on a much larger scale which eventually develop vaccines and various treatments. However, continuous cell lines are not used in virus production for human vaccines as these are derived from malignant tissue or possess malignant characteristics.

B. Virus cultivation and study

Cell culture is widely used for the propagation of viruses as it is convenient, economic, and easy to handle compared to other animals. It is easy to observe cytopathic effects and easy to select particular cells on which the virus grows as well as to study the infectious cycle. Cell lines are convenient for virus research because cell material is continuously available. Continuous cell lines have been extremely useful in cultivating many viruses previously difficult or impossible to grow.

C. Cellular and molecular biology

Cell culture is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging), the effects of different toxic compounds on the cells, and mutagenesis and carcinogenesis. The major advantage of using cell culture for any of these applications is the consistency and reproducibility of results that can be obtained from using a batch of clonal cells.

D. In Cancer Research

Normal cells can be transformed into cancer cells by methods including radiation, chemicals, and viruses. These cells can then be used to study cancer more closely and to test potential new treatments.

E. Gene therapy

Cells having a non-functional gene can be replaced by cells which are having the functional genes, for which the cell culture technique is used.

F. Immunological studies

Cell culture techniques are used to know the working mechanism of various immune cells, cytokines, lymphoid cells, and the interaction between disease-causing agents and the host cells.

G. Others

Cell lines are also used in in-vitro fertilization (IVF) technology, recombinant protein, and drug selection and improvement.