

Review On: Drug Evaluation Methods and Animal Cell Culture

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ABSTRACT : The drug evaluation method is a comprehensive framework crucial for assessing the safety, efficacy, and overall performance of pharmaceutical substances. This systematic approach encompasses preclinical studies, clinical trials, and post-marketing surveillance. Preclinical studies involve in vitro and in vivo testing to evaluate safety and pharmacokinetics. Clinical trials progress through phases, from initial safety assessments to large-scale trials confirming efficacy. Endpoints and biomarkers play a pivotal role in measuring outcomes. Regulatory approval processes ensure thorough evaluation before market entry. Post-marketing surveillance monitors long-term safety and effectiveness. Health economic evaluations assess costeffectiveness, and real-world evidence complements clinical trial findings. The significance of these methods lies in safeguarding patient safety, informing clinical decisions, advancing scientific knowledge, and fostering public confidence in pharmaceuticals.

Keywords : In vivo and in vitro testing, Clinical trials, Patient safety,Efficacy of drugs, Post – Marketing surveillance monitors

I. INTRODUCTION :

Drug evaluation methods are crucial in assessing the safety, efficacy, and overall performance of pharmaceutical substances. These methods aim to provide a systematic approach to gathering data and making informed decisions about drug development and approval. Key components include preclinical studies, clinical trials, and post-marketing surveillance. The integration of diverse methodologies ensures a comprehensive understanding of a drug's effects, helping to safeguard public health and advance medical science.The importance of drug evaluation methods is multifaceted and crucial for various stakeholders involved in healthcare, pharmaceutical development, and public safety. Here are key aspects highlighting their significance:

i)Patient Safety:Drug evaluation methods are paramount in identifying potential risks and adverse effects, ensuring the safety of individuals receiving the medications.Rigorous evaluation helps prevent the introduction of harmful or inadequately tested drugs into the market

ii)Efficacy Confirmation:These methods provide a systematic approach to determining the effectiveness of drugs, ensuring that they deliver the intended therapeutic benefits.Accurate assessment of efficacy is essential for informed medical decision-making and patient care.

iii)Regulatory Approval:Regulatory agencies use evaluation data to make informed decisions regarding drug approval and market authorization.Stringent regulatory processes contribute to public confidence in the safety and efficacy of approved pharmaceuticals.

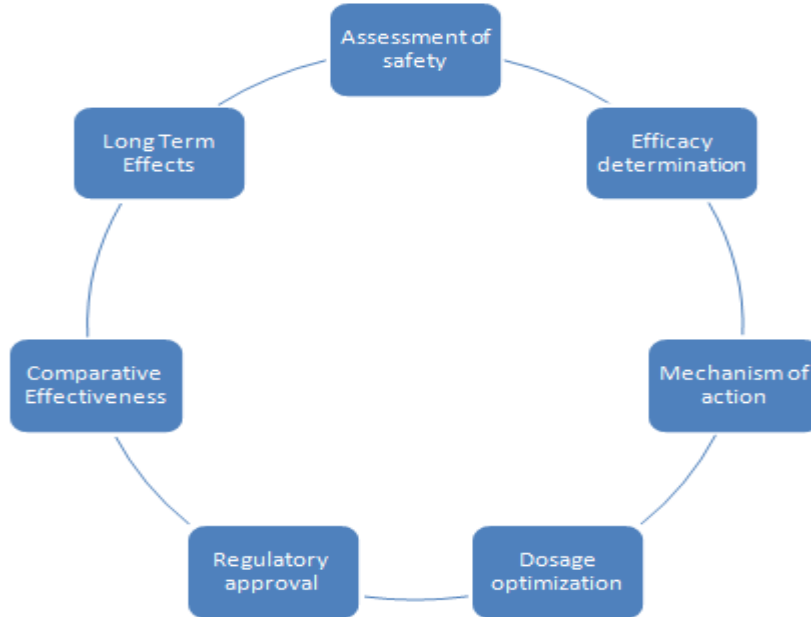
iv)Clinical Decision Support:Healthcare professionals rely on evaluation findings to make informed decisions about the most appropriate and effective treatments for their patients.Comprehensive evaluation data guide evidence-based clinical practices

v)Innovation and Scientific Advancement:Drug evaluation methods contribute to scientific knowledge by providing insights into disease mechanisms and potential treatment avenues.Continuous refinement of evaluation techniques drives innovation in drug development and healthcare.

vi) Public Health Impact:Effective drug evaluation safeguards public health by ensuring that medications used in healthcare systems contribute positively to patient outcomes.Mitigates the risk of widespread harm from ineffective or unsafe drugs.

vii) Ethical Considerations:Rigorous evaluation methods uphold ethical standards in research and healthcare, prioritizing patient well-being and ensuring the ethical treatment of study participants.Balancing risks and benefits is essential in ethical drug development and clinical research.

➤ **Aim And Objectives:**



➤ **SCREENING PHARMACOLOGICAL ACTIVITIES :**

• **ANALGESIC ACTIVITY:**

Definition: The drugs which are used to relieve pain are called as Analgesic Drugs.

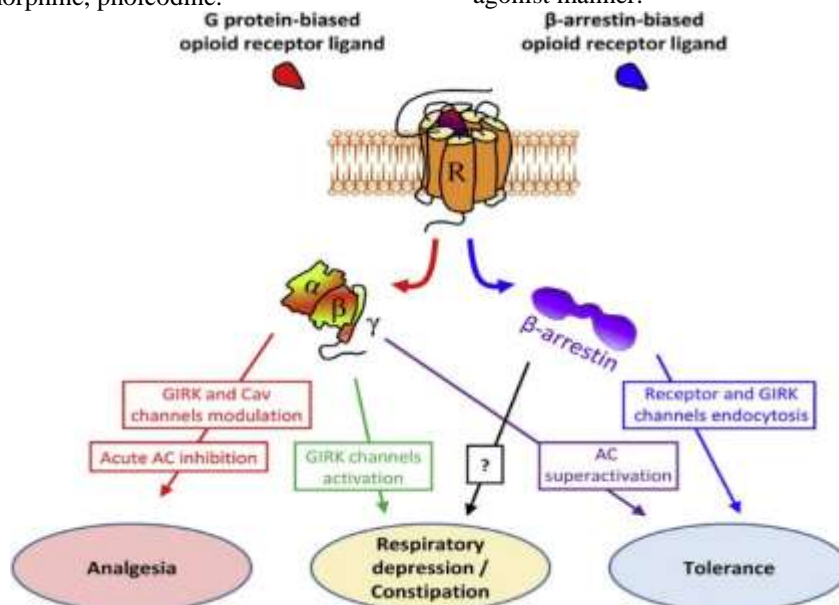
Classification:

- 1) Opioid μ receptor agonists:
 - (i) Natural opium alkaloids: Morphine, codeine.
 - (ii) Semi synthetic opioids: Diacetylmorphine (heroin), ethyl morphine, pholcodine.

- (iii) Synthetic opioids: pethidine, methadone, fentanyl, Remifentanyl, Tramadol, Tapentadol.

• **Mechanism of action:**

G-protein coupled receptors are bound by opioid agonists, which hyperpolarize cells. In order to cause analgesia, the majority of therapeutically useful opioid analgesics bind to MOP receptors in the central and peripheral nervous systems in an agonist manner.



Screening Method For Analgesic Activity:

1) In Vitro screening model:

Bioassay Of Nociceptin: Take the tissue from male Swiss mice, guinea pigs, Sprague Dawley rat and New Zealand albino rabbit. Suspend in 10 ml organ bath containing krebs solution

oxygenated with 95% O₂ and 5% CO₂. Set the temperature around 33°-37°C and apply 0.3-1 g of resting tension. Stimulate the tissue with two platinum ring electrodes. Measure the electrically evoked contraction isotonicity with a strain gauge transducer and record on multichannel chart recorder. After equilibration period of about 60 min the contraction induced by electrical field stimulation become stable ; at this time perform the cumulative concentrations response curve to nociceptin or opioid peptide. Perform four electrical field stimulation with each tissues at 30 min intervals. Add agonist and antagonist to bath. The biological effect of application of agonist or antagonists are expressed as % inhibition of electrical filed stimulation-induced contraction. Contractile response to electrical field stimulation are expressed as % increment to spontaneous activity of the tissue.

2) In vivo Screening model:

Hot Plate Method: The hot plate test was used to measure response latency time. Animals were placed on hot metal plate maintained at 55°C. Elapsed time between placement of the animal on the hot plate and the occurrence of the licking of the hind paws, shaking, or jump off from the surface was recorded as response latency in seconds. The specificity and sensitivity of the test were Increased by measuring the reaction time of the first evoked behaviour regardless of whether it is paw licking or jumping. The responses were measured at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 24 hours. Only mice that showed initial nociceptive

response within 30 seconds were selected and used for the study. The cut-off time for the hot plate latencies was set at 30 secs. The percentage protection against thermal pain stimulus was calculated according to the following formula:

$$\text{Percentage protection Against Thermal Stimulus} = \frac{\text{Test mean (Ta)} - \text{Control mean (Tb)}}{\text{Control Mean (Tb)}} * 100$$

• ANTIHYPERGLYCEMIC ACTIVITY:

Definition: Drugs used in diabetes treat diabetes mellitus by altering the glucose level in the blood is known as Antihyperglycemic drugs.

Classification:

1. Enhance Insulin secretion:
 - (a) Sulfonylureas (KATP Channel blockers):
 - (i) First generation: Tolbutamide
 - (ii) Second generation: Glibenclamide (Glyburide), Glipizide, Gliclazide, Glimepiride
 - (b) Meglitinide/phenylalanine analogues: Repaglinide, Nateglinide .
 - (c) Glucagon-like peptide-1 (GLP-1) receptor Agonists (Injectable drugs): Exenatide, Liraglutide
 - (d) Dipeptidyl peptidase-4 (DPP-4) inhibitors: Sitagliptin, Vildagliptin, Sitagliptin, Alogliptin, Linagliptin
2. Overcome Insulin resistance:
 - (a) Biguanide (AMPK activator): Metformin
 - (b) Thiazolidinediones (PPAR γ activator): Pioglitazone
3. Miscellaneous antidiabetic drugs:
 - (a) α -Glucosidase inhibitors: Acarbose, Miglitol, Voglibose
 - (b) Amylin analogue: Pramlintide
 - (c) Dopamine-D2 receptor agonist: Bromocriptine
 - (d) Sodium-glucose cotransport-2 (SGLT-2) Inhibitor: Dapagliflozin.

Mechanism Of Action :

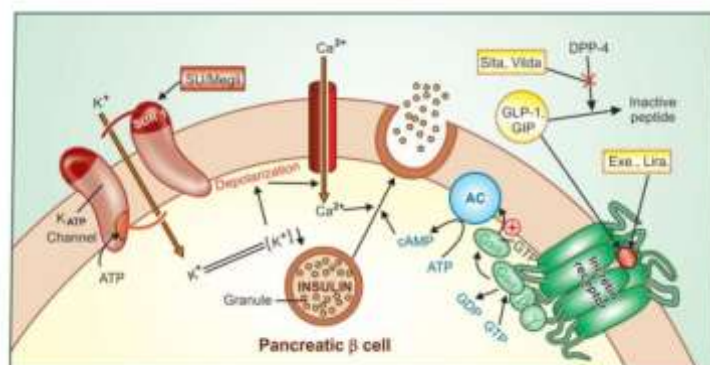


Fig. 19.6: Mechanism of action of insulin secretagogues

Screening method of Antihyperglycemic activity:

1) In-Vitro screening model:

Inhibition of α -glucosidase activity: The α -glucosidase enzyme inhibition activity was performed by incubating α -glucosidase enzyme solution with phosphate buffer which contains test samples of different concentrations at 37°C for 1 hr in maltose solution. The reaction mixture was kept in boiling water for a few minutes and cooled. Glucose reagent was added and its absorbance was measured at 540 nm to estimate the amount of liberated glucose from maltose by the action of α -glucosidase enzyme. The percentage of inhibition and IC50 was calculated.

2) In-Vivo Screening model:

Dithizone Induced Diabetic Model: Dithizone is an organosulfur compound, it has chelating property. Dithizone is used in induction of diabetes in experimental animals. In dithizonised diabetic animals, the levels of zinc, iron, and potassium in the blood were found to be higher than normal. Dithizone permeates membranes and complex zinc inside liposomes, then release of protons, this enhances diabetogenicity.

➤ ANIMAL CELL CULTURE TECHNIQUE :

Cell culture is the process by which human, animal, or insect cells are grown in a favorable artificial environment. The cells may be derived from multicellular eukaryotes, already established cell lines or established cell strains. Animal cell culture is now one of the major tools used in the life sciences in areas of research that have a potential for economic value and

commercialization. The development of basic culture media has enabled scientists to work with a wide variety of cells under controlled conditions; this has played an important role in advancing our understanding of cell growth and differentiation, identification of growth factors, and understanding of mechanisms underlying the normal functions of various cell types.

Biology Of Cultured cell:

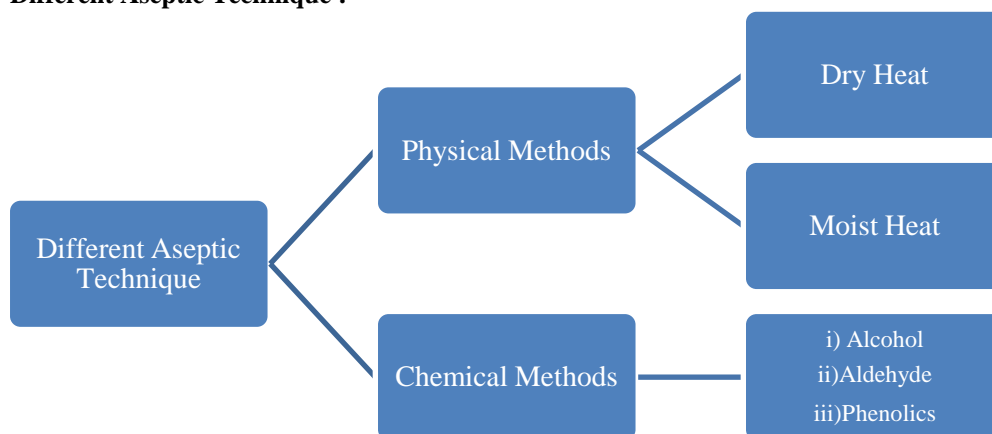
1. Cell adhesion.
2. Cell proliferation.
3. Cell differentiation.
4. Metabolism of cultured cells
5. Initiation of cell culture.
6. Evolution and development of cell lines.

Equipment used in animal cell culture lab:

1. Sterile Work Area/Cell culture hood (i.e., laminar-flow hood or biosafety cabinet)
2. Incubator (humid CO2 incubator recommended)
3. Water bath
4. Centrifuge
5. Refrigerator and freezer (-20°C)
6. Cell counter (e.g. Automated Cell Counter or hemocytometer)
7. Inverted microscope
8. Liquid nitrogen (N2) freezer or cryostorage container
9. Sterilizer (i.e., autoclave) Aseptic refer to the a procedure that is performed under sterile conditions.

The aim of aseptic technique is to prevent the access of micro-organisms during preparation and testing.

• Different Aseptic Technique :



- **Culture Vessels :**

1. Aspiration pump (peristaltic or vacuum)
2. pH meter
3. Roller racks (for scaling up monolayer cultures)
4. Confocal microscope
5. Flow cytometer
6. Cell culture vessels (e.g., flasks, Petri dishes, roller bottles, multiwell plates)
7. Pipettes and pipettors
8. Syringes and needles
9. Waste containers
10. Media, sera, and reagent
11. Cells
12. Cell cubes

Media Development:

Culture media are provided in dehydrated or powdered form, in liquid concentrate, or as working solutions. Dehydrated media for broth cultures need to be prepared by dissolving in distilled water and adjusting the final pH prior to sterilization. Powdered media for agar cultures must be dissolved in distilled water, stirred, then boiled or autoclaved prior to pouring into sterile petri dishes using aseptic techniques. Culture media provided as liquid concentrates must be aseptically diluted in distilled water, adjusted for pH, and dispensed into sterile containers for storage. Culture media provided as working solutions are ready to use after the addition of any required serum or other supplements under sterile filtration and/or aseptic conditions, and warming to the appropriate temperature for cell growth.

Serum-Free Media Development and Sterilization:

Serum-free media are media designed to grow a specific cell type or perform a specific application in the absence of serum. The use of serum-free media (SFM) represents an important tool, that allows cell culture to be done with a defined set of conditions as free as possible of confounding variables.

Advantages of using serum-free media:

- (i) Increased definition.
- (ii) More consistent performance.
- (iii) Easier purification and downstream processing.
- (iv) Precise evaluations of cellular function.
- (v) Increased growth and/or productivity.
- (vi) Better control over physiological responsiveness.

The constituents of culture media, water and containers contribute to the contamination by vegetative cells and spores. The media must be free from contamination before use in fermentation. Sterilization of the media is most commonly achieved by applying heat and to a lesser extent by other means (physical methods, chemical treatment, and radiation). Sterilization includes – Heat sterilization, Radiation, Chemical methods, physical methods etc.

- **Contamination :**

Microbial contamination is a major issue in cell culture, but there are a range of procedures which can be adopted to prevent or eliminate contamination. Contamination may arise from the operator and the laboratory environment, from other cells used in the laboratory, and from reagents. Some infections may present a risk to laboratory workers: containment and aseptic technique are the key defence against such risks. Remedial management of suspected infection may simply mean discarding a single potentially infected culture. However, if a more widespread problem is identified, then all contaminated cultures and associated unused media that have been opened during this period should be discarded, equipment should be inspected and cleaned, cell culture operations reviewed, and isolation from other laboratories instituted until the problem is solved. Attention to training of staff, laboratory layout, appropriate use of quarantine for new cultures or cell lines, cleaning and maintenance, and quality control are important factors in preventing contamination in cell culture laboratories.

- **Cryo-preservation :**

It is the process of freezing cells and tissues using liquid nitrogen to achieve extremely low temperatures with the intent of using the preserved sample to prevent the loss of genetic diversity. Semen, embryos, oocytes, somatic cells, nuclear DNA, and other types of biomaterial such as blood and serum can be stored using cryopreservation, to preserve genetic materials. The primary benefit of cryopreservation is the ability to save germplasm for extended periods, therefore maintaining the genetic diversity of a species or breed. There are two common techniques of cryopreservation: (i) slow freezing and (ii) vitrification. Slow freezing helps eliminate the risk of intracellular ice crystals. If ice crystals form in

the cells, there can be damage or destruction of genetic material. Vitrification is the process of freezing without the formation of ice crystals.

(i) Slow freezing: During slow freezing, cells are placed in a medium which is cooled below the freezing point using liquid nitrogen. This causes an ice mass to form in the medium. As the water in the medium freezes, the concentration of the sugars, salts, and cryoprotectant increase. Due to osmosis, the water from the cells enters the medium to keep the concentrations of sugars, salts, and cryoprotectant equal. The water that leaves the cells is eventually frozen, causing more water to diffuse out of the cell. Eventually, the unfrozen portion cellular becomes too viscous for ice crystals to form inside of the cell.

(ii) Vitrification: The second technique for cryoconservation is vitrification or flash freezing. Vitrification is the transformation from a liquid to solid state without the formation of crystals. The process and mechanics of vitrification are similar to slow freezing, the difference lying in the concentration of the medium. The vitrification method applies a selected medium which has a higher concentration of solute so the water will leave the cells via osmosis. The medium is concentrated enough so all of the intracellular water will leave without the medium needing to be reconcentrated. The higher concentration of the medium in vitrification allows the germplasms to be frozen more rapidly than with slow freezing. Vitrification is considered to be the more effective technique of freezing germplasms.

Cytotoxicity:

Cytotoxicity refers to the ability of a substance to cause damage or death to cells. In the context of research and medicine, it is often assessed to understand the potential harmful effects of drugs, chemicals, or treatments on living cells. Various assays and tests are employed to measure cytotoxicity and evaluate the impact of substances on cell viability.

• Methodology : In vitro culture

Embryonic organ culture is an easier alternative to normal organ culture derived from adult animals. The following are four techniques employed for embryonic organ culture.

• **Plasma clot method:** The following are general steps in organ culture on plasma clots.

1. Prepare a plasma clot by mixing 15 drops of plasma with five drops of embryo extract in a watch glass.

2. Place a watch glass on a pad of cotton wool in a petri dish; cotton wool is kept moist to prevent excessive evaporation from the dish.

3. Place a small, carefully dissected piece of tissue on top of the plasma clots in watch glass.

The technique has now been modified, and a raft of lens paper or rayon net is used on which the tissue is placed. Transfer of the tissue can then be achieved by raft easily. Excessive fluid is removed and the net with the tissue placed again on the fresh pool of medium.

• **Agar gel method:** Media solidified with agar are also used for organ culture and these media consist of 7 parts 1% agar in BSS, 3 parts chick embryo extract and 3 parts of horse serum. Defined media with or without serum are also used with agar. The medium with agar provides the mechanical support for organ culture. It does not liquefy. Embryonic organs generally grow well on agar, but adult organ culture will not survive on this medium. The culture of adult organs or parts from adult animals is more difficult due to their greater requirement of oxygen. A variety of adult organs (e.g. the liver) have been cultured using special media with special apparatus (Towell's II culture chamber). Since serum was found to be toxic, serum-free media were used, and the special apparatus permitted the use of 95% oxygen.

• **Raft Method:** In this approach the explant is placed onto a raft of lens paper or rayon acetate, which is floated on serum in a watch glass. Rayon acetate rafts are made to float on the serum by treating their 4 corners with silicone. Similarly, floatability of lens paper is enhanced by treating it with silicone. On each raft, 4 or more explants are usually placed. In a combination of raft and clot techniques, the explants are first placed on a suitable raft, which is then kept on a plasma clot. This modification makes media changes easy, and prevents the sinking of explants into liquefied plasma.

• **Grid Method:** Initially devised by Trowell in 1954, the grid method utilizes 25 mm x 25 mm pieces of a suitable wire mesh or perforated stainless steel sheet whose edges are bent to form 4 legs of about 4 mm height. Skeletal tissues are generally placed directly on the grid but softer tissues like glands or skin are first placed on rafts, which are then kept on the grids. The grids themselves are placed in a culture chamber filled with fluid medium up to

the grid; the chamber is supplied with a mixture of O₂ and CO₂ to meet the high O₂ requirements of adult mammalian organs. A modification of the original grid method is widely used to study the growth and differentiation of adult and embryonic tissues.

- **Uses:** Cultured organs can be an alternative for organs from other (living or deceased) people. This is useful as the availability of transplantable organs (derived from other people) is declining in developed countries. Another advantage is that cultured organs, created using the patient's own stem cell, allows for organ transplants where the patient would no longer require immunosuppressive drugs.

- **Tissue culture:**

Tissue culture is a technique in which fragments of plants are cultured and grown in a laboratory. Techniques: Following are the different types of tissue culture techniques:

(i)Seed Culture: In this culture, the explants are obtained from an in-vitro derived plant and introduced into a laboratory where they proliferate. The explant should be sterilized to prevent it from tissue damage.

(ii)Embryo Culture: This involves the in-vitro development of an embryo. For this, an embryo is isolated from a living organism. Both, a mature or an immature embryo can be used in the process. Mature embryos can be obtained from ripe seeds. The immature embryos are obtained from the seeds that failed to germinate. The ovule, seed or fruit is

already sterilized; therefore, it does not need to be sterilized again.

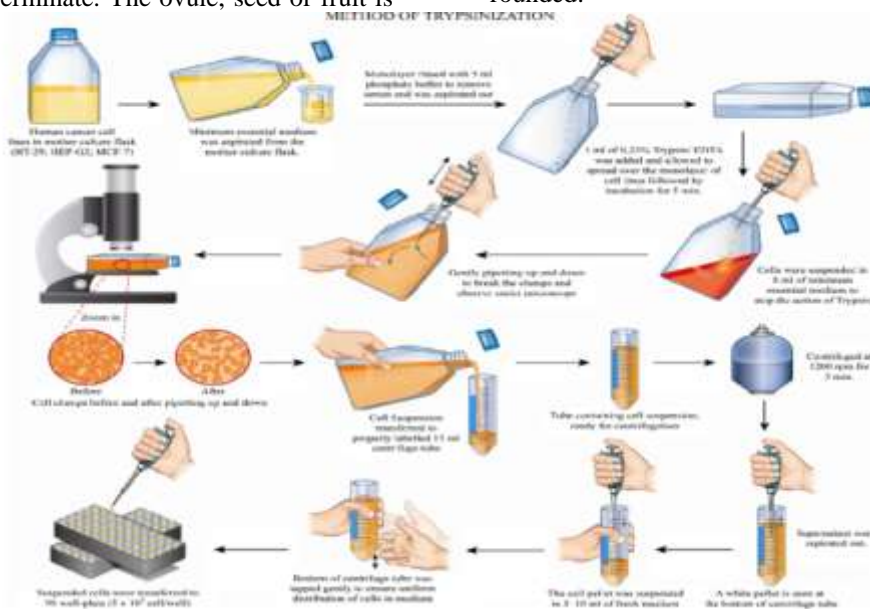
(iii) Callus Culture: A callus is an unorganized, dividing mass of cells. When the explants are cultured in a proper medium, the callus is obtained. The growth of callus is followed by organ differentiation. The culture is grown on a gel-like medium composed of agar and specific nutrients required for the growth of the cells.

(iv) Organ Culture : In this, any organ of the plant such as shoot, leaf, can be used as an explant. A number of methods can be used for the organ culture, such as plasma clot method, raft method, grid method, and agar gel method. This method is used to preserve the structure and functions of an organism.

(v)Protoplast Culture: It is a cell without a cell wall. A protoplast can be cultured using the hanging-drop method, or micro-culture chambers. In protoplast culture, a number of phases can be observed: development of cell wall, cell division, regeneration of a whole plant.

➤ **Trypsinization:**

Trypsinization is the process of cell dissociation using trypsin, a proteolytic enzyme which breaks down proteins, to dissociate adherent cells from the vessel in which they are being cultured. When added to cell culture, trypsin breaks down the proteins that enable the cells to adhere to the vessel. Trypsinization is often used to pass cells to a new vessel. When the trypsinization process is complete the cells will be in suspension and appear rounded.



➤ **Cell Separation :**

Cell separation, also commonly referred to as cell isolation or cell sorting, is a process to isolate one or more specific cell populations from a heterogeneous mixture of cells. There are a variety of methods used to isolate cell populations, usually based on one or more properties unique to that cell type (size, electric charge, density, shape, protein expression, etc.).

(i) Fluorescence-Activated Cell Sorting (FACS): Fluorescence-activated cell sorting (FACS) technology labels cells with fluorescent markers based on internal or external characteristics of the targeted cell group. Then, the cells are identified and sorted one at a time based on the color of the marker.

(ii) Density Gradient Centrifugation: Density gradient centrifugation is a common cell isolation technique where the biological sample is centrifuged until the cell types are isolated into layers depending on their respective densities.

(iii) Magnetic-Activated Cell Sorting (MACS): Magnetic-activated cell sorting (MACS) binds magnetic particles to cells through an antibody interaction with surface markers of the targeted cells. The cell group is then magnetically isolated from the rest of the biological sample.

(iv) Filtration: Filtration is a cell isolation method based on cell size. Using a filtration device specific to the targeted cell group's size, smaller cells pass through the device while larger cells are trapped.

v) Aptamer-Based Cell Isolation: Aptamer-based cell isolation uses aptamers such as DNA or RNA to bind to targeted cells based on specific biomarkers.

(vi) Buoyancy-Activated Cell Sorting (BACS™): Buoyancy-activated cell sorting (BACS™) is a revolutionary cell isolation technology. Microbubbles bind to cells, and the targeted cells are removed from the biological sample through flotation.

➤ **Phases of cell division :**

(i) Interphase: It is the process through which a cell must go before mitosis, meiosis, and cytokinesis. Interphase consists of three main phases: G1, S, and G2. G1 is a time of growth for the cell where specialized cellular functions occur in order to prepare the cell for DNA replication.

(ii) Prophase: Prophase is the first stage of division. The nuclear envelope is broken down in this stage, long strands of chromatin condense to form shorter more visible strands called chromosomes, the nucleolus disappears, and microtubules attach to the chromosomes at the disc-shaped kinetochores

present in the centromere. Microtubules associated with the alignment and separation of chromosomes are referred to as the spindle and spindle fibers. Chromosomes will also be visible under a microscope and will be connected at the centromere. During this condensation and alignment period in meiosis, the homologous chromosomes undergo a break in their double-stranded DNA at the same locations, followed by a recombination of the now fragmented parental DNA strands into non-parental combinations, known as crossing over. This process is evidenced to be caused in a large part by the highly conserved Spo11 protein through a mechanism similar to that seen with topoisomerase in DNA replication and transcription.

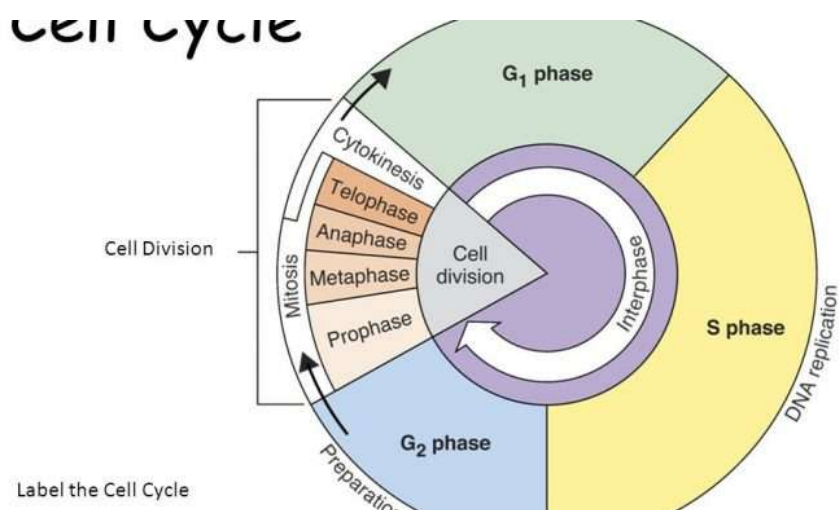
(iii) Metaphase: In metaphase, the centromeres of the chromosomes align themselves on the metaphase plate (or equatorial plate), an imaginary line that is at equal distances from the two centrosome poles and held together by complexes known as cohesins. Chromosomes line up in the middle of the cell by microtubule organizing centers (MTOCs) pushing and pulling on centromeres of both chromatids thereby causing the chromosome to move to the center. At this point the chromosomes are still condensing and are currently one step away from being the most coiled and condensed they will be, and the spindle fibers have already connected to the kinetochores. During this phase all the microtubules, with the exception of the kinetochores, are in a state of instability promoting their progression toward anaphase. At this point, the chromosomes are ready to split into opposite poles of the cell toward the spindle to which they are connected.

(iv) Anaphase: Anaphase is a very short stage of the cell cycle and it occurs after the chromosomes align at the mitotic plate. Kinetochores emit anaphase-inhibition signals until their attachment to the mitotic spindle. Once the final chromosome is properly aligned and attached the final signal dissipates and triggers the abrupt shift to anaphase. This abrupt shift is caused by the activation of the anaphase-promoting complex and its function of tagging degradation of proteins important toward the metaphase-anaphase transition. One of these proteins that is broken down is securin which through its breakdown releases the enzyme separase that cleaves the cohesin rings holding together the sister chromatids thereby leading to the chromosomes separating. After the chromosomes line up in the middle of the cell, the spindle fibres will pull them apart. The chromosomes are split

apart while the sister chromatids move to opposite sides of the cell. As the sister chromatids are being pulled apart, the cell and plasma are elongated by non-kinetochore microtubules.

(v) Telophase: Telophase is the last stage of the cell cycle in which a cleavage furrow splits the cells cytoplasm (cytokinesis) and chromatin. This occurs through the synthesis of a new nuclear envelope

that forms around the chromatin gathered at each pole. The nucleolus reforms as the chromatin reverts back to the loose state it possessed during interphase. The division of the cellular contents is not always equal and can vary by cell type as seen with oocyte formation where one of the four daughter cells possess the majority of the duckling.



Label the Cell Cycle

Application of animal cell culture in Drug evaluation / Discovery:

Cell culture techniques play a crucial role in drug discovery by providing a controlled environment to study the effects of potential drugs on cells.

This includes:

- (i) **Target Identification and Validation:** Cell cultures help identify and validate drug targets by studying the behavior of specific cells in response to various stimuli
- (ii) **Screening Compounds:** High-throughput screening of drug compounds on cell cultures allows for the rapid identification of potential drug candidates and their effects on cellular functions
- (iii) **Toxicity Testing:** Cell cultures are used to assess the toxicity of drug candidates, ensuring their safety and minimizing adverse effects before advancing to clinical trials.
- (iv) **Mechanism of Action Studies:** Understanding how drugs interact with cells at a molecular level is vital. Cell cultures enable researchers to investigate the mechanisms of action of potential drugs.
- (v) **Disease Modeling:** Culturing cells from diseased tissues helps researchers create models for various diseases, facilitating the

study of disease progression and drug responses in a controlled setting.

- (vi) **Studying Cellular Signaling Pathways:** Cell cultures allow researchers to explore signaling pathways affected by drugs, aiding in the development of targeted therapies.
- (vii) **Patient-Derived Models:** Culturing cells from patients allows the creation of personalized models, improving the relevance of drug testing and potentially leading to more effective treatments.
- (viii) **Optimizing Drug Formulations:** Cell cultures are used to optimize drug formulations, ensuring stability and bioavailability before moving to more complex animal or human studies.

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