Chapter

A Brief Concept of Cell Culture: Challenges, Prospects and Applications

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Abstract

Cell culture is an *in vitro* technique in which cells, tissues, or organs (animal origin) are artificially grown with the support of an artificial environment that encompasses culture medium, CO_2 level, pH indicator, temperature keeping tissues alive and growing appropriately. Organ culture, Primary explant culture, and Cell culture among them cell culture widely used for the understanding of cell growth, normal functions, identification of growth factors, viral vaccine development, recombinant DNA (rDNA) technology, and immunobiological research. Due to high feasibility, cell culture practices highly demandable in the pharmaceutical industry. As well as animal cell culture used in laboratory research to study the cytotoxicity of new drug metabolic studies, aging, therapeutic proteins, the effects of drugs and toxic compounds on the cells and mutagenesis and carcinogenesis. There are a lot of issues in cell culture, Mycoplasma is one of the major. During cell culture, a single antibiotic often cannot kill the mycoplasma. Besides, culture media, pH indicator, incubation, cryopreservation, thawing, passaging of cells, and trypsinization have a great impact on cell culture. This chapter will help the reader to understand the whole process of cell culture and its applications, which will take them one step forward in their virology and cell culture research along with inspiration. This chapter also aids in the concept of cell count, cell suspension, CCF measurement, MOI (Multiplicity of Infection), and cell infection. Eventually, the reader will get a crystal clear concept of cell culture.

Keywords: Chicken Embryo Fibroblast Culture, BHK-21, Cell maintenance, Culture media, Cell culture, Passaging of cells, Cell harvest, Antibiotics, Bulk antigen, Quality control, Vaccine candidate development

1. Introduction

Cell culture or Chicken embryo fibroblast cell culture is a fundamental laboratory technique that widely used in virology, vaccinology, molecular biology, microbiology as well as in biotechnology field. In this *in vitro* study controlled environment like media, pH, CO_2 level, temperature, humidity, O_2 flow mimics *in vivo* condition for cell to grow. Within last past decades a few species have been considered *in vitro*

utilizing cell culture technique exposing essential data regarding their biology. The chicken or avian embryo gives an amazing model for the study embryology, developmental biology and production of pharmaceutical proteins in transgenic chickens moreover take part in virology, immunization advancement as well as vaccinology research. Chicken embryo fibroblast cell culture has great impact on veterinary vaccine and biochemical production research. Besides, chicken stem cells have given a perfect opportunity for producing cell-based transgenic birds and a powerful source of cells for vaccine production for poultry and human viral diseases. Besides, they give knowledge into fundamental science namely drug sensitivity testing, cell tracking and cytotoxicity testing [1]. However, various important virus or viral diseases have never been explored at a cellular level so that cell culture technology is still in an early stage of its potential progress. In this document, it is summarized the basic steps engaged in the establishment of primary cell culture and concept of cell culture technique to serve as a practical guide for current and future researcher to leverage the power of cell cultures. This approach has the potential to produce valuable outcomes and suggestions regarding CEF cell culture, cell metabolism, adaptation to different stresses and challenges. Although most published papers discussed on CEF cell culture, BHK-21 and other cell lines, here focused on basic concept and necessary measurement for primary cell culture that inevitably helpful for novice as well as experienced researchers or laboratory personnel of this field [2]. Physiological and physiochemical condition: Nature and impact of pH level, temperature, concentration of O_2/CO_2, and osmotic pressure (culture media) that can be altered the result of the study as well as their effects on the cell culture [3]. Cell culture technology has spread productively within a century; a variety of culture media has been designed. This chapter goes through the brief concept, challenges and current issues of cell culture or chicken embryo fibroblast cell culture technique. Current article does not substance to be a complete guide but rather helps the researcher to plan and make consonant decision to their research experimental or work.

2. Cell culture

General concept of cell culture is the propagation of cells or fibroblast or living tissues in a defined media that conducive their growth. Shortly, a growth of cell artificially known as cell culture. CEF (Chicken Embryo Fibroblast) cell culture is the culture of fibroblast cells obtained from embryo. Embryonated eggs commonly used in the production of bulk antigens, vaccines and other biochemical. SPF (Specific Pathogen Free) eggs obtained from SPF (Specific Pathogen Free) chicken flocks which have been intensively monitored for infectious agents and have not been vaccinated; or, where justified (e.g. for production of some inactivated vaccines) and in line with the marketing authorization, from healthy chicken flocks. For the propagation of virus laboratory personnel or researcher should have to choose specific route of inoculation of the SPF egg based on the study microorganisms (virus) that is being propagated [4].

Baby Hamster Kidney cells (BHK-21) are generally used in life science research work and the biopharmaceutical industry. Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, mouse myeloma cells comprising NS0 and SP2/0, hybridomas, and human cell lines (HEK293, HT-1080) are the very commonly used mammalian cell lines at large scale [5]. Among these cell lines, BHK-21 has found applications at large scale in veterinary viral vaccines against foot and mouth

disease (FMD) and rabies virus, as well as heterologous protein production (Factor VIII) [6]. The BHK-21 cell line was established in 1961 from the kidneys of 5 Syrian hamsters from litter number 21. Since this time, this cell line has been considered as a research facility standard for the development of countless viruses and the study of numerous biological processes. Baby hamster kidney (BHK) cells are one of many different vertebrate cell types used for the propagation of viruses by infection and transfection [7]. Generally obtained from BHK cells can vary widely if not obtained from the ATCC. For the production of animal products (vaccine) baby hamster kidney (BHK) cells are generally used, the most important of which is the production of a vaccine against FMD and rabies. Also, BHK was used in the production of recombinant proteins, such as blood coagulation factor VIII for the extraction of DNA from Pseudorabies virus and production of capture antigen enzyme-linked immunosorbent assay (ELISA) when diagnosing Japanese Encephalitis (JE) [8–10]. BHK-21 cells were grown and propagated in modified minimum essential media (MEM) by adding a set of proteins including Lactalbumin (2.50 g/l), yeast extract (1.00 g/l), peptone (2.50 g/l), New Zealand casein (1.00 g/l); Glutamine (0.50 g/l) and 2% sodium bicarbonate were also added to the culture medium. Also, 5–10% of the serum treated with polyethylene glycol 6000 was added to modified MEM. Penicillin G and streptomycin was added at the rate of 100 IU to control microbial load. After keeping the BHK cells in a liquid nitrogen tank and passing them through the preparation steps, the cells were transferred to CCF for subsequent cultivation. The flasks were kept in incubators at an operating temperature of 36°C or 36.5°C or 37°C and 5% carbon dioxide. Maintaining the starting pH of the culture ranged from 7.1 to 7.2 or 7.4 while carbon dioxide was used to control the pH. Common morphology of some cells are *Fibroblastic (or fibroblast-like) cells*: Shape- bipolar or sometimes multipolar, elongated, that attached to a substrate during growth and wrench commonly during culture. *Epithelial-like cells*: Shape-polygonal, Dimensions-regular, and which attached to a substrate in discrete patches. *Lymphoblast-like cell*: Shape-spherical, Commonly, grown in suspension and do not attach to the surface of flask.

3. Basic steps of cell culture technique

Cell culture or CEF cell culture is crucial laboratory work. During cell culture laboratory personnel or researchers have to maintain chronological workflow step by step. By maintaining the following steps cell culture should be done very smoothly. For CEF cell culture, SPF eggs were collected from authentic sources with a legal document. Then incubate for the recommended date. Afterward-

1. Personal hygiene and safety management

2. Sterilization and disinfection

3. Arrangement of instrument and appliances as well as power supply

4. Biosafety cabinet (BSC) management

5. Management of essential ingredients and chemicals like Media, FBS, Trypsin, Phosphate Buffer Saline (PBS), Tincture of iodine, Antibiotics, etc.

6. Management of Pipettes, Burette, Tips, Waste disposal box, Aspirator, Tissue
7. Chopping, washing embryo, and filtering (for CEF cell culture)
8. Centrifugation
9. Cell suspension and stock management
10. Cell count and splitting of the cell
11. CCF, Media, and incubator management
12. Cell line development
13. Cell observation, harvest
14. Cell count, subculture, infection, and bulk antigen production

4. Fundamental parts of cell culture technology

4.1 Culture medium

Culture medium is a composition of nutrients and selected buffer that helps to grow an organism naturally. Media can be designed based on variety of cell, types of cells because it is necessary for cell survival, proliferations and growth. The influence of cell culture technology creates inevitable progress in molecular biology research. This technique widely utilized in different fields like the assessment of toxicity and efficacy of new drugs, development of various biopharmaceutical products and vaccines, and used in reproductive technology. No one probably would argue against the claim that a culture medium is the foremost essential measure in cell culture technique. Selection of suitable media for research goal is essential. Sometimes researchers should modify a properties or composition of medium in order to their experiment. There are mainly two types of media used by researcher such as natural media and synthetic media [3, 11, 12]. *Natural media*: The media that comprising of natural biological substances, e.g. embryo extract, plasma, and serum. Coagulant or clots i.e. plasma which is separated from heparinized blood, serum, and fibrinogen. Tissue extracts and Extracts of chicken embryos, liver, and spleen and bone marrow extract considered as a natural cell culture media. Biological fluids such as Plasma, serum, lymph, amniotic fluid, and pleural fluid also used as culture media. *Synthetic media*: The media comprising with basal medium and supplements, like-serum, growth factors, and different hormones. On the basis of type of supplements synthetic media can be classified into a few bunches such as: (1) Serum-containing media: In this media as a supplement bovine, human, equine, or other serum is used. This media causes less reproducibility and microbial contamination due to unknown and unclear media composition. (2) Serum-free media: This media has a high reproducibility due to presence of the supplements of bovine serum albumin or α-or β-globulin (crude protein fractions). (3) Xeno-free Media: Prepared based on human-source components i.e. human serum albumin, are commonly used as supplements but there are no

animal components used as supplements. (4) Protein-free media: Prepared based on undefined components like- peptide fractions (protein hydrolysates) which are generally used as supplements, and (5) Chemically defined media: Undefined components are used as supplement. Crude protein fractions, tissue extracts, hydrolysates are not good supplements, but highly purified components (recombinant proteins) are appropriate supplements. Followings are some brand name of culture media commonly used in the laboratory: Eagle's Minimum Essential Medium (EMEM) a simpler basal medium (BME) for culturing mouse L cells and HeLa cells. Dulbecco's Modified Eagle's Medium (DMEM) is a basal media (BME). Iscove's Modified Dulbecco's Medium (IMDM) used for growth of lymphocytes and hybridomas. Hybri-Care Medium is a combination and modification of DMEM considered for the propagation of hybridomas and other fastidious cell lines. Ham's Nutrient Mixtures used for Chinese hamster ovary (CHO) cells. Kaighn's modification of Ham's F-12 (Ham's F-12 K) was designed to develop and differentiation of primary cells with or without serum. DMEM/F12 Medium is a 1:1 mixture of Dulbecco's modified EMEM and Ham's F-12 for the growth of a wide range of cell types in both serum and serum-free formulations. Leibovitz's L-15 Medium for biopsy samples without CO_2.

4.2 pH value

pH plays a vital role in cell culture. The cell growth rate is decline associated with Fluctuations in pH level. That's why routine monitoring is essential. For the cells is 7 the optimal pH, and decline or increase in pH can hinder the growth of cells. More decrease in pH level (usually in between 6.0–6.5), can stop the growth rate of the cells, and cells are start losing viability at low pH level. That's why pH level should be maintained and monitored carefully for individual cell line. If pH level fall rate is less than 0.1 units/day, that indicates the cell condition is good and no need to hurry to change the culture medium immediately. If it is 0.4 units/day (pH drop rate), that indicates the culture medium need to be changed quickly [13]. Alkali (like- NaOH, KOH) or acid (HCl) solution helps to control pH level in culture medium. Besides, $NaHCO_3$ (sodium bicarbonate) or natural buffer solution, and addition (need base) of CO_2 gas to the bioreactor also helps to maintain optimum pH level in culture medium. Generally, pH electrode (silver chloride electrochemical-type) used within the bioreactor [14]. For the proliferation of cell in culture medium an optimum, stable as well as balanced pH is essential. Depending upon cell type and culturing process pH level may vary and specific. Generally, 5–10% CO_2 required to grown cells using buffered media that contain $NaHCO_3$ (sodium bicarbonate) and where maintained the range of pH 7.2 to 7.4 [15]. CO_2 incubator, optimum pH level, ideal temperature, optimum moisture condition, sterile and clean working environment are essential to maintain and complete an experiment. In the cell culture medium, the carbonate buffer helps to hold constant pH and take parts in releasing CO_2 gas in the CO_2 incubator. Color of the culture medium depending and changing with the pH level of culture medium. Color indicates altering the medium and CO_2 levels [3]. Commonly, 4–10% of CO_2 is practiced in the cell culture technique. By maintaining HCO_3^- concentration and CO_2 tension in culture medium one can easily achieved optimum pH and osmolality [16]. By observing the color of the media can easily identify the pH condition like- Phenol red to yellow/ orange color indicates too acidic where pH 6.8 (bellow), Red to pink color indicates pH above 7.0 to 7.7 which is normal, and bright fuchsia color indicates pH 8.0 to 8.2 (too alkaline).

4.3 Effect of temperature

Generally, cell culture need 37°C for incubation called control temperature. Proliferation and multiplication of cells are significantly decreased at more than 40°C, like 41°C or 42°C temperature, and increased temperature may also cause high apoptotic rate of CEF cells. Cell viability, apoptosis, proliferation, and oxidative status of cells in culture medium can be altered with high temperature. ROS (reactive oxygen species) formation increased with increasing Temperature (Proportionally) [17]. During the transportation must be care full about temperature. Besides regular or routine inspection is recommended for better results. In cell culture technique one of the most challenging issues is to grow cell, that's why an ideal temperature play a vital role in cell culture along with good supplementation of nutrients. For the cell division the optimum temperature should be vary on cell type that assist to maintain growth rate. Generally, at optimal temperature metabolic function of cell is optimum as well as good that helps to increase their size, and proliferation rate [18]. Temperature requirement varies based on cell type like- *S. frugiperda* (sf-21, *Trichophusia ni* (Tn-5), *S. frugiperda* (sf-9) (insect) need 27°C, Chinese Hamster Ovary (CHO) (Mammalian)-need 36–37°C, Human embryonic kidney (HEK- 293 T) (Human) need 37°C, HF 205 and HF 210 (plant) need 27°C.

4.4 Incubation

A device in which microbial culture or cell culture is grown and maintained with customized temperature, humidity (relative humidity 95%), oxygen, CO_2 level, and other conditions. For virus cultivation, cell culture as well as cell infection, vaccine development incubation is a very much crucial and fundamental issue. The incubator is an essential instrument in cell biology research, Microbiology, Biotechnology, Molecular biology research. On the other hand, an egg incubator is one of the most important for embryonated egg production in the laboratory and large scale. BOD (Biological Oxygen Demand) incubator is popular in this regard. There are so many necessary points always bear in mind to maintain good incubation. Firstly, cleaning inside, cabinets, outside and handle that helpful to eradicate cross-contamination, also helps to hold a good quality of cell, media, SPF (Specific Pathogen Free) eggs, and other chemicals or ingredients. Logbook maintenance is another vital issue to maintain high-quality research work. Through logbooks, laboratory personnel can easily identify any issue related to the incubator. A dedicated power supply essential to maintain cell quality, growth, metabolism as well as important to maintain cell physiology. CO_2 level and the supply are very much essential to maintain moisture and pH (normal range is 7.2 to 7.4). Generally, 5% CO_2 with 37°C is used to maintain the cell. Eventually, inventory management will helpful for GMP (Good Manufacturing Practice) and proper cell culture as well as laboratory work.

4.5 Cryopreservation

Cryopreservation is the method in which intact living cells are conserved as intact in liquid nitrogen at cryogenic temperatures. On the other hand, in cryopreservation system using low temperature helps to protect living cells structurally intact. Freezing system keeps the living cells frequently for a long time (often for years). Freezing temperature ceased their typical metabolic activity that's why cells are protected from damage caused by long time preservation, and chemical reactivity. Gently handle

cells because cell may be damaged and will get stress during the freeze–thaw process. Optimal cryopreservation of cells relies on proper freezing and thawing methods. A successful cryopreservation method calculating based on recovery rate of cells (frozen) from low temperature, percentage of alive cells, and rate of cells that function as normal after thawed [19]. Basically, for cryopreservation harvest the cells in exponential growth. Then gentle centrifugation done at 125 × g for 10 minutes. After that check and scree the media. The procedure starts with taking freezing medium (GM-which warm at room temperature for 30 minutes) that containing a cryoprotectant such as Dimethyl sulphoxide (DMSO) (e.g. 5–10% v/v DMSO), fetal bovine serum (FBS) (10–20% v/v) and at high cell density (1–5 × 10^6/ml) and sometimes added knock-out serum replacer (KoSR; 20% v/v final), bovine serum albumin (5% final) or human serum albumin. From some recent research [20–22] it is said that freezing rate has great impact on viability of cryopreserved cells. It is suggested that cells be slowly cooled (like 2°C, −20°C, −80°C for at least 24 hours and finally preserved in liquid nitrogen at −196°C) that gives better surviving rate of cell in the cryopreservation process. Record logs must maintain during all the steps. From the final cryovial (that contain cell at −196°C) remove one vial and restore the cells in culture medium to determine cell viability and sterility. Recovery rate of cryopreserved cells depends on the types of cultured cell. Some cell needs several days, some shows low viability on the day of culture, in some cases cell produce debris, some cells are shows normal viability after 24 hours' post-thaw. Before retrieving of cryopreserved cells clean the biosafety cabinet, prepare the CCF, media, FBS and arrange all the instruments and appliances (sterile). Then wash the cryovial with 70% ethanol and place it in a water bath for 2 to 5 min at 37°C to melt. Transfer the thawed cell in a tube and gentle centrifugation (10 minutes at 125 × g) needs to discard (supernatant) cryoprotectant in the meanwhile collect the cell pellet and suspend the cells in 1 mL or 2 mL of complete growth medium (GM) then proceed for cell count and subculture in new CCF for 24 hours' observation. In the process of cryopreservation, significant rate of cell survival and maintenance of cell integrity (structural and morphological) can be achieved by using cryoprotective agents (CPAs). Excipient is an ingredient added intentionally to the drug substance which should not have pharmacological properties in the quantity used. Commercially available CPAs namely Dimethyl sulphoxide (DMSO) is commonly used as CPA. Factors behind the success of cell survival [23] are: (a) Type and concentration of cryoprotectants (an additive, such as glycerol or dimethyl sulphoxide, that can protect cells against freezing injury). (b) Cell density in cryopreservation solution at the time of freezing. (c) Cooling and thawing rates of cell suspension. (d) Dilution rate of thawed cell suspension. The main advantages [24] of cryopreservation are easily found original cell lines from the safety stocks, preserve the cells for year after year and lastly smoothly perform continuous research or experiments.

4.6 Thawing

Correctly thawing of cells is crucial to recover quickly, yielding the highest viability and functionality possible. Some cryoprotectants (e.g. DMSO) has toxic effect on cells, due to the possibility of toxicity cells should be thawed rapidly and not allowed to remain in the freezing medium no longer than required time. Firstly, retrieve the cryovial containing the frozen cells from liquid nitrogen (−80°C or – 196°C freezer) and immediately place it into a 37°C water bath (for 1 to 2 min) or place immediately in a pre-equilibrated thermo-conductive rack or tube module resting on dry ice to

minimize cell warming/thawing. Rapidly thaw the cells (< 1 minute) by carefully swirling the cryovial in the 37°C water bath up to there is a little bit of ice left within the cryovial. Then place the cryovial into a BSC. Gently wipe the outside of the cryovial with 70% IPA (Isopropyl Alcohol) prior to open the cryovial screw. Carefully add (dropwise) required amount of pre-warmed GM into the tube (centrifuge) that containing recently thawed cells. Place the cell suspension in centrifuge machine for centrifugation and set 200 × g for 5–10 minutes (it may vary based on cell type). Inspect the transparency of supernatant and visibility of a pellet at the bottom after completing the centrifugation. Discard the supernatant aseptically without breaking the pellet. Softly resuspend the cells by gently pipetting with GM and prepare required concentration, then transfer the cell suspension into the CCF, and place it in the suggested culture environment. Inspect the cells using an inverted microscope for morphology. Examine an aliquot of cells for the ability to exclude trypan blue. If cells pass both inspections, they are ready for culture [25].

4.7 Passaging of cells

Subculture of cell commonly known as passaging of cells and the ratio of subculture is 1:2. The main concept of passaging: cells are split into half in each subculture. Continuous cell lines should will be passaged with higher split ratio due to their higher replication rate. Usually the number of times the cells have been subcultured into a new CCF known as passage number. In the case of diploid cell cultures, the number cell passage is partially equal to the number of population doubling level (PDL) since the culture was begun. On the other hand, PDL of continuous cell lines is not fixed like diploid cell culture. Mostly the PDL is an estimation or prediction. PDL may ups and down with cell stress and cell death (due to necrosis, apoptosis). Loss of proliferation capacity of cell, contamination of culture medium may also responsible. A common formula for the calculation of population doubling level: PDL = 3.32 (log Xe – log Xb) + S; where Xb is the cell number at the beginning of the incubation time, Xe is the cell number at the end of the incubation time, S is the starting PDL. Another common formula used to calculate the population doubling level is [26]: Log (*N/N*) x 3.33**;** Where N is the number of cells in the culture vessel at the end of a certain time interval, and N is the original number of cells plated in the vessel, Population doubling and passage number are often mixed up or thought to mean the same thing. The passage number describes the number of times that a culture has been subcultured The population doubling time is important to know when the number will be double and passaging will be required. According to ATCC [24] the population doubling time calculate with the formula: DT = T ln2/ln(Xe/Xb); where T is the incubation time in any units, Xb is the cell number at the beginning of the incubation time, Xe is the cell number at the end of the incubation time.

4.8 Trypsinization

Trypsin is an enzyme that is used to remove adherence proteins from a cell surface. Generally, trypsin-based disaggregation so-called trypsinization. Disaggregation of cells from the CCF commonly crude trypsin used, the effect of raw (crude) trypsin can easily be neutralized by commercially available serum (FBS) or trypsin inhibitor. On the other hand, pure trypsin is also used in the cell degradation process which is less toxic and very specific in action [27]. Commonly

0.05% trypsin used in laboratory work. Sometimes trypsinization causes cell damage and sometimes may not effective for some cells thus other dissociation agents (enzyme) are recommended for the dissociation of cells. Warm and cold trypsinization are the two common approaches. An extensively used method is warm trypsinization. In which cells are washed with basal salt solution and then add warm trypsin (37°C) adequately and stirred properly. The supernatant dissociated, the cells are dispersing in the medium. In the case of cold trypsinization, cellular damage is reduced, resulting in a high yield of viable cells also improved survival rate. For cold trypsinization cells are maintained in ice after washing with media or salt then treated with cold trypsin for 6–24 h. After that remove and discard the trypsin and incubate the CCF at 37°C (for 20–30 min). Dispersion of cells may start and fully dispersed cells counted using hemocytometer then dispersing in a medium for further use. The easiest way of trypsinization is a). Discard the media from CCF b). Wash the cell surface with PBS (4 ml for 75 cm^2 CCF) c). Take trypsin (Room temperature) d). Rinse monolayer of cell with trypsin–EDTA (2 to 4 ml for 75 cm^2 CCF) e). Stay for 2 minutes then discard trypsin and Incubate the CCF at 37°C for 5 minutes f). Tapping the CCF and collect the cells by scrapper stored in a tube g). Spin down the cells, resuspended by adding the growth medium or fresh medium, and Count the cells h). Split into a new flask and Incubate at 37°C.

4.9 CCF measurement

CCF denotes a Cell Culture Flask. It is also known as a tissue culture flask. CCF is important for culturing of cells, transportation of cells, and media. There are a lot of different volumes of CCF used for research work. Commonly, used flask volume are 25 cm^2, 75 cm^2, 175 cm^2, 225 cm^2, 300 cm^2. Commonly 75 cm^2 CCF preferable for laboratory work. Cell contamination generally appeared during cell culture laboratory. Proper knowledge of CCF handling can minimize cross-contamination, which improves the quality and physiology of the cell. Rough handling of CCF during media transfer, passaging, scrapping will be responsible for the different vital issues. Mycoplasma contamination is one of them. Prompt and Improper pipetting during cell harvest and split from CCF may cause stressful conditions on cells and resulting in cell death. Scrapping of the cell for subculture or infection or cell count gently handles the CCF, corkscrew. Mild flame spark on corkscrew (CCF) by flame gun or gas burner helpful to safe the cell and flask environment. Covering the cork with Paraflim very much essential to save the cell. IPA (Isopropyl Alcohol) spray must be done before and after handling of CCF. After application of IPA, then CCF, media, FBS (Fetal Bovine Serum), tips, flame gun, trypsin other materials, and appliances allow entering into BCS (Biosafety Cabinet) for further processing. Some points should be bear in mind regarding CCF such as, is CCF allow pipettes, tissue scrapper, tissue spatula properly? Is CCF has marked on both sides? Is there any leakage? Is there any crake on cork? Is the bag of CCF tightly pack? Is the CCF clean (inside)? These points might be helpful for cell culture.

4.10 Cell suspension

The cell suspension is nothing but suspension culture. It's another type of cell culture where a small amount/volume of cells is permitted to grow in growth media forming suspension called cell suspension. If the cells are derived from other cultures

or homogenized tissue, then use suspension culture. Both suspension culture and adherent culture are the same.

4.11 Cell count and cell infection

Cell counting was performed using a hemocytometer (Neubauer improved counting chamber, Precicolor HBG, Germany) or MacMaster slide and Trypan blue exclusion every 24 h (1,1 mixture of 0.2% Trypan blue in normal saline solution and sample). After placing the stock cell suspension on the hemocytometer and place a coverslip on it. Count cell of 4 (16 × 4) site, then the average of 4 sites of hemocytometer and count the total cell as = Average (number of the cell) × 10,000 × 2. Cell culture flask such as 25 cm^2 contain 5 to 10 ml culture media, 75 cm^2 contain 10 to 30 ml culture media, and 175 cm^2 contain 40 to 150 ml culture media. One (1) cm^2 need 90,000 cells likewise 75 cm^2 need 75 × 90000/5 = 13,50,000 cells minimum. Viable cells are considered as unstained ones and while stained cells are considered as dead under the inverted microscope. Cell counts are important for monitoring cell health and proliferation rate, assessing immortalization or transformation, seeding cells for subsequent experiments, transfection or infection, and preparing for cell based assays. Cell counts must be accurate, consistent, and fast, particularly for quantitative measurements of cellular responses. Cell infection is required for virus propagation, bulk antigen production. MOI rate is very much essential in cell infection. There are three types of MOI commonly used in the laboratory such as 1 MOI, 0.1 MOI, 0.001 MOI where 1 MOI means one (1) virus can infect one (1) cell. 0.1 MOI denotes 10 cells infected by one (1) virus and one virus can infect 100 cells in 0.001 MOI. Generally, practice 0.1 MOI means one virus is enough to infect 10 cells.

4.12 PFU (PFU), ELD_{50}, EID_{50}, MOI, $CCID_{50}$, $TCID_{50}$

Plaque forming units (pfu) is an assessing of the total number of infectious virus particles. It is ascertained by a plaque-forming assay. In the field of virology study, a plaque-forming unit (PFU) is a measurement of the number of particles capable of forming plaques per unit volume i.e. virus particles. It is a functional measurement rather than a measurement of the absolute quantity of particles: viral particles that are defective or which fail to infect their target cell will not produce a plaque and thus will not be counted. For instance, a solution of virus with a concentration of 1,000 PFU/µl indicates that 1 µl of the solution contains enough virus particles to produce 1000 infectious plaques in a monolayer cell, but no inference can be made about the relationship of pfu to the number of virus particles.

ELD_{50}- Embryo Lethal Dosage. ELD_{50} unit is the amount of virus that will kill 50 percent of inoculated eggs.

EID_{50}- Embryo Infective Dosage. EID_{50} unit is the amount of virus that will infect 50 percent of inoculated eggs.

Multiplicity of infection (MOI) is the average number of virus particles infecting each cell. MOI is related to pfu by the following formula: Multiplicity of infection (moi) = Plaque forming units (pfu) of virus used for infection/number of cells. For example, if $2x10^6$ cells is infected by 50 ml of the virus with a titer of 10^8 pfu/ml. The moi will be $0.05*10^8/2*10^6 = 2.5$. The fraction of cells that are not infected is $P(0) = 1 - e^{-moi}$. To ensure 99% of cells are infected requires moi > 4.6. Assume the conditions used for plaque assay and TCID assay do not alter the expression of infectious virus.

$TCID_{50}$/ml and pfu/ml are related by pfu/ml = 0.7 * $TCID_{50}$. As a working estimate, one can use pfu/ml = 0.5 * $TCID_{50}$ [28].

$CCID_{50}$: Cell culture infectious dose which will infect 50% of the cell.

$TCID_{50}$ is the tissue culture infectious dose that will infect 50% if the cell monolayers are challenged with the defined inoculum. Two methods commonly used to calculate $TCID_{50}$ (can also be used to calculate other types of 50% endpoint such EC_{50}, IC_{50}, and LD_{50}) are a). Spearman-Karber [29] b). Reed-Muench method.

4.13 Plaque assay

Plaque-based assays are the standard method used to determine virus concentration in terms of infectious dose. Viral plaque assays determine the number of plaque-forming units (pfu) in a virus sample, which is one measure of virus quantity. This assay is based on a microbiological method conducted in Petri dishes or multi-well plates like 6 well or 24 well etc. Specifically, a confluent monolayer of host cells is infected with the virus at varying dilutions and covered with a semi-solid medium, such as agar or carboxymethyl cellulose, to prevent the virus infection from spreading indiscriminately. A viral plaque is formed when a virus infects a cell within the fixed cell monolayer [30]. Virus quantification involves counting the number of viruses in a specific volume to determine the virus concentration. In research and development (R&D) based commercial and academic laboratories, the production of viral vaccines, recombinant proteins using viral vectors, viral antigens and clone screening, multiplicity of infection (MOI) optimization, and adaptation of methods to cell culture all require virus quantification. For quantification of virus incubated after infection at 37°C in a 5% CO_2 incubator at 6, 12, 24, 36, 48, 60, and 72 h post-inoculation (hpi) based on the requirement to visualize plaques in wells [31, 32]. To quantify virus there are a lot of other methods used such as Focus forming assay (FFA), Endpoint dilution assay, Protein assays, Hemagglutination assay (HA), Bicinchoninic acid assay, Single radial immunodiffusion assay, Transmission electron microscopy (TEM).

4.14 Cell harvest

Knowledge of splitting, media, trypsinization, cell handling is essential to harvest cells. Firstly, remove and discard the media from CCF. For 75 cm^2 CCF, mild PBS wash is required before the application of warm trypsin or trypsinization process. Add trypsin (2–4 ml/75 cm^2) then incubate as like trypsinization process. When detached cells appear then add 2–5 ml growth media (GM) to inactivate trypsin. Gently pipette to disperse the medium to ensure recovery of >95% of cells. Sometimes commercial trypsin inhibitor is added. Carefully centrifuge the collected cell suspension at 300–1000 X g for 5–10 min. Discard the supernatant and add GM at the required amount for the preparation of cell count. Split the cells after counting or go for further processes that need. There is a lot of problems that may be appeared like detachment difficulty of cells from culture flasks, cell adherence difficulty, insufficient attachment of cells, low viability of cells, clumping after detachment, damage of cell membrane, and cell death. The possible solutions to the above problems will be careful during the following such as a). Check the quality, date, and concentration of trypsin before use b). Be careful during antibiotic application if any c). Splitting, media replacement required before harvesting if cells are in stress d). Avoid vigorous pipetting and long centrifugation.

4.15 Antibiotics

Routine cell and tissue culture according to good cell culture practice (GCCP) [33] should not require the use of antibiotics as they can never be relied on as a substitute for effective aseptic techniques. However, its use is still widespread e.g., OECD TG 432 [34] due to established routine procedures in many laboratories. Antibiotics are agents that may arrest or disrupt fundamental aspects of cell biology, and, while they are effective against prokaryotic cells (i.e. Bacteria), they are also capable of causing toxic effects in animal cells. Not surprisingly, antifungal agents, being directed at higher order, eukaryotic microorganisms, are likely to be more toxic to animal cell cultures. In addition, antibiotics often make it more difficult to detect microbial contamination. These obvious contraindications, the use of antibiotics in cell and tissue culture should be focused in two areas: a) Protection of materials at high risk of contamination such as tissues, organs, and primary cultures in cases where sterility cannot be guaranteed, and b) The positive selection of recombinant cell clones based on the expression of antibiotic resistance genes [33]. If antibiotics are needed, a justification for the use of antibiotics in the procedure is suggested.

4.16 Laboratory management

Good laboratory setup is essential for cell culture as well as good laboratory practice is also essential for better and smooth work. The following discussion and points are very much important for a cell culture laboratory and are also supported by Maneesha et al. [35] and Coecke et al. [36].

Location and ideal layout with the purpose-built facility is the first priority. Room data sheet (RDS) in which available all the data of every facility, specification of the room define its location, a number of doors, windows, pass box, ventilator, light, air conditioner, fire alarm like everything present on that room permanently. Specification of instruments must be available at the working area that helps the laboratory personnel to operate it. Standard operating procedure (SOP) helps to do research/ laboratory work in a defined way to get a better outcome. Define the area of a room with a specific class and biosafety level by the standard of ISO, GMP. Define or specify works of laboratory personnel with defined working areas according to CDC, NIH-USA also beneficial for a good outcome. There are four biosafety levels based on hazard or pathogenicity or virulence of microorganism and toxicity of agents. The basic biosafety level known as biosafety level 1 (BSL-1). In this level generally very common research work done with easy protection. Normally in BSL-1 working with those organisms or agents which are not harmful to healthy person. In biosafety level 2 (BSL-2) working with those organisms or agents which is known as moderate-risk agents, and known as a potential threat to human resulting produce disease (by ingestion or through percutaneous or mucous membrane exposure) of varying severity. Generally, cell culture should be performed at BSL-2 laboratory. Sometimes the biosafety level depends on the type of cell line and working style. In biosafety level-3 (BSL-3) working with that agents which have the capability to transmit through air (aerosol transmission). BSL-3 agents may be indigenous or exotic, having potential threat to human, may cause serious health issue, and may be potentially lethal. Biosafety level-4 (BSL-4) deals with exotic agents that create life-threatening disease of an individual through infectious aerosols and for which no treatment is available. These organisms or exotic agents are restricted to high containment laboratories. The easily accessible facility of the emergency shower should be available in the laboratory area. Access

control in laboratories should be helpful to maintain unwanted occurrences. A separate logbook is very much helpful for liquid nitrogen and CO_2 management. Ventilation and pressure control like negative and positive pressure controlled area must be defined, HEPA filtered providing positive pressure to clean areas, is recommended where space and resources allow. Electricity room (uninterrupted power supply (UPS) units should be provided for essential equipment (class II cabinets, incubators, air filtration) and to allow cell culture procedures to be completed. Accountability for all the staff will provide a smooth working environment. Documentation, Training (Fumigation, 5 s, SOP, etc.) and Monitoring of staffs, Emergency service provider contract with the third party are essential for good laboratory management. Before receive and entering all the reagents, chemicals, and supplier's documents must be checked by maintaining a logbook. For the management of inventory and documents should be established hard copy or electronic form that stored the information of materials, cells, suppliers, overall all the possible information. Staff safety is a vital issue. The primary concerns regarding safe management of liquid nitrogen storage are frostbite burns from skin contact with liquid nitrogen and asphyxiation due to exposure to low oxygen levels when nitrogen gas is released from vessels. Finally, all service personnel entering the laboratory should receive instruction in special laboratory hazards and any necessary procedures for working in clean areas e.g. gowning, hand disinfection. All the person needs to use a separate biohazard bag/bin to ensure safe hazard management. Like-Infectious non-sharp waste (incineration/deep burial)-Yellow bag; Plastics and sharps (chemical treatment/autoclaving/shredding/microwaving)- Blue bag; Infectious non-sharp waste (chemical treatment/autoclaving/microwaving)-Red bag; Incineration ash and solid chemical wastes (secure landfill)- Black bag.

4.17 Challenges during passage of cell

During cell culture, laboratory personnel and researcher should follow up the cell routinely. Splitting of the cell depends on cell doubling number, cell type, pH level, media, and so many cell culture-related issues. There is a lot of challenge situation faced by laboratory workers. The most common problems that cause major issues in the laboratory may also ruin the running works are misidentification of cell line, Contamination of culture and media, Rough handling, Poor cell growth, Poor cell attachment, Improper trypsinization during harvesting, Improper cell count, Improper split ratio during the passage, Clumping of cell, Cell death. Incubation time, temperature, and CO_2 level also have a great impact on cell culture as well as on subculture.

4.18 Maintenance of cell

The routine follow-up of cell morphology is necessary. To maintain a good cell line routinely change of the medium is essential for the both proliferating or non-proliferating cells. The culture medium should be changed repeatedly in the case of proliferating cells compared to the non-proliferating one. The rate of cell growth, cell morphology and metabolism of cell indicates the urgency and time interval of medium change. For example, HeLa cells are rapidly growing transformed cells, in the case of HeLa cell the culture medium should be changed twice within 7 days, whereas for slowly growing non-transformed cells (like IMR-90 cells) the culture medium may be changed once in a week. Continuous cell lines, Chicken embryo fibroblast cell (CEFC), Embryonic cells and transformed cells develop quickly that's

why these cells need rapid sub-culture and altering the culture medium. While normal cells are grow slowly. Generally altering the medium depends on pH level. Immediately change whole medium when the pH level appeared 7.0, cells are stop proliferating at pH 6.5, and the cells may lose their durability and viability when the pH level drop in between 6.5 to 6.0. The drop rate of pH is commonly estimated for each and individual cell line with a selected culture medium. If the drop rate of pH is less than 0.1 units/day, that indicates no harm and no need to hurry to change the culture medium immediately. When the drop rate of pH is 0.4 units/day, that indicates the culture medium need to be changed immediately [13]. A laboratory person or a researcher can easily maintain cells by maintaining SOP of cell handling and culture procedure, by counting passaging time because 10–30% density of cell is standard but at 80–90% density cell should be split as well as cell count may be helpful in this regard. Must pay attention to media quality, color, clarity, foul smell results from infection of the cell. Cell health and cell concentration, appearance observed regular interval by bright field microscope with 20–60x magnification may help to maintain cells and eradicate clumping, detachment, apoptosis. Quality control (QC) documents, Certificate of Analysis (COA), Cell transportation SOP are crucial to maintaining cells. Logbook entry for all the daily activities like pH level daily basis, media condition, temperature, CO_2 level, assigned peoples information, cell condition, passage number, all the information about the cell very much essential to maintain cell for either small or large scale work.

4.19 Mycoplasma: a issue

Mycoplasma contamination is a serious and widespread problem in cell culture. Mycoplasma is often passed from culture to culture and from lab to lab. Mycoplasma can ruin whole research if data collected from mycoplasma-infected cells or cultures. Among all the contaminants (biological) in the laboratory mycoplasma have the capability to spread rapidly and causes detrimental effect on cells because of their detection rate is very low as well as their serious impact on cell lines. In spite of the fact that mycoplasmas are actually microscopic organisms (like bacteria) but they have some particular characteristics that make them identical. Mycoplasmas can easily survive and multiply at high densities without producing any noticeable signs. They are very harmful to any cell culture. Mycoplasmas can easily alter the host cells' metabolism and morphology, cause chromosomal aberrations and damage of cell that provoke cytopathic effects. Mycoplasma ought to be tested at least once a month is recommended in laboratory and research work. Two different testing methods, such as DAPI staining and PCR are helpful but a commercially available mycoplasma kit is also recommended for the detection [37]. A routine screening process might be helpful to eradicate mycoplasma contamination from the lab. The following points are essential to prevent mycoplasma issues [38]. a). Wearing personal protective equipment (PPE) during cell culture that includes a dedicated, clean lab coat and gloves b). Checking the COA, QC pass of cells' origins c). Ensure proper sterilization d). Always clean the working area e). To avoid cross-contamination work with only one cell at a time f). Always ensure covering the media bottles and CCF. Do not use the hood for storage and work always within biosafety cabinet g). Should be cautious in the use of antibiotics because it is reported that antibiotics have no impact on mycoplasmas h). Maintain logs for record-keeping that help to identify possible contamination sources if needed i). SOP develop for routine mycoplasma screening.

4.20 Bulk antigen and vaccine candidate development

A large scale of virus production is known as bulk antigen production. A Proper culture of the cell, passaging, infection of cell-based on required MOI by a specific virus, harvesting, filtration of the virus, QC test, and COA gives the final confirmation regarding the virus and the process. After formulation, dosing should be done with the help of bulk antigen that helps to develop proposed vaccine candidate based on reference manual, and guidelines.

5. Cell culture procedure

CEF cell culture is widely used by researchers in the biopharmaceutical industry and veterinary vaccine production. The following steps should be maintaining chronologically to develop primary cell culture from the chicken embryo.

1. SPF eggs are incubated and collect the embryonated egg at 8–11 days (need base).

2. Cleaning the outside of egg by tincture of iodine, Cracking the egg into BSC.

3. Collect the embryo by forceps, place it into a sterile Petri dish, and chopping the body parts (embryo) with scissors.

4. Cut off head, wings, legs, remove the visceral parts and wash (PBS) rest of the body in Petri dish until clean the blood.

5. Chopping the clean body with scissors and gently pipette and aspirate by syringe.

6. Collect the suspension into a sterile falcon tube and perform mild centrifugation at 300 rpm for 5 min.

7. Add 0.25% trypsin EDTA to suspend the pellet by gentle pipetting (Recommendation: for 12 embryos add 10 ml trypsin and 10 ml PBS for suspension).

8. Centrifuge the suspension at 600 to 1000 rpm for 5 to 10 min (at 37°C).

9. Collect the supernatant into a new sterile falcon tube and filter it with double layer sterile gauze and collect it into a new tube and add 10 to 15 ml GM into it for 12 embryos. Generally, add GM two times (1:2) of collected cell/fluid.

10. Wash it by centrifugation at 10,000 rpm, 25°C for 10 min, and discard the supernatant. Then add GM to reconstitute and gently pipette. Repeat this step 2 times and collect the pellet (Suspected that 12 embryos produce 2 ml pellet).

11. Add 13 ml GM with the collected pellet (2 ml) and gently mix 15 ml cell suspension consider as a stock cell suspension.

12. Count the cell based on the stock with the help of a hemocytometer then split the incorporation of the cell with growth media that contain 10% FBS based on CCF measurement.

13. Observe the cell distribution within the CCF under an inverted microscope.

14. Place the CCF into a CO_2 incubator at 5% level, pH 7.2 to 7.4, 37°C for overnight. Then observe the cell morphology under a microscope.

6. Application of cell culture

Nowadays application of cell culture is exceptionally essential in life science and medical science. Cell culture technique is an excellent tool its applications are [1, 39, 40] as a). Production of pharmaceutical biochemicals b). Embryological study (CEF cell culture) c). Recombinant biomaterials (rDNA) and vaccine manufacturing, testing of the drug, drug sensitivity, and cytotoxicity of cell d). Production of human and animal vaccines (primary chicken fibroblast cells e). Manufacturing of immunotherapy f). Production of different enzymes, hormones (synthetic), immunobiological (like monoclonal antibodies, interleukins, lymphokines), and anticancer agents g). Cell culture is an excellent way to teach cell biology study h). Production of agricultural products like milk, (cultured) meat, fragrances i). In the microbiological study (Virus propagation, virology) j). Genetic engineers and biotechnologist are utilizing it within their field of research k). The aging, toxic compound study, cell morphology, cell physiology, and the study of mutagenesis cell culture have great impact.

7. Cell handling, storage and challenges

There are a lot of measures that should be taken to handle cells smoothly. Smooth and gentle handling of the cell gives better outcomes. An SOP must be defined clearly in the handling procedure of a cell. Clean area, BSL, pressure control of the cell culture room, personal hygiene, general laboratory management, gowning, skilled manpower can play a vital role in this regard. Generally, cell incubate at 36–37°C in a 5% CO_2 sometimes CO_2 level 4–10%, and time required based on cell types, research methodology. For the short preservation (few hours <2 hours) of the cell need normal freezing and for about day-long preservation required −20°C with growth media or cryoprotectant agent. Cryopreservation is required for the yearlong storage of cells. The temperature logbook is an essential document in the lab. Any fluctuation, problems in power supply, user entry of fridge/ cold room, and incubator are inevitably helpful to handle and maintain cells. Besides lock, access control or password system in laboratory, incubator, and fridge might be helpful in this regard. Resulting in easily identify the problems if any. Vigorous pipetting during cell harvesting, splitting cause cell damage sometimes causes cell death. A few toxic and harmful substances are eluted from the microfilters during sterilization. That's why practicing the sterile technique strictly and selecting cultural instruments carefully. It is recommended that washing all the instruments with the culture medium immediately before performing cell culture. Nevertheless, other types of contamination happened from plastic instruments or trace elements, even in water that affects the cells in culture [11]. Viruses, bacteria, mycoplasma, and endotoxins contamination may appear due to rough handling and haphazard performance. Strict environmental control is necessary to check cross-contamination in cell culture [41].

8. Quality control, GMP and cGMP

Quality control (QC) is known as maintaining the quality and authenticity of anything related to the research or laboratory work like cell, media, PBS, Polymerase chain reaction (PCR), or RT-PCR report. That's why the QC department, as well as personnel are important. Every life science researcher ought to know and aware of the current Good Manufacturing Practice (cGMP), QC process and system. QC, GMP, and cGMP have the same objectives and more or less same activities. In GMP and cGMP all are the followings clearly defined- Rules, regulations, and guidelines; good implementation; all aspects of the product's lifecycle and manufacturing process; product development and raw materials selection to the final production process; testing; storage, and shipment. The worlds policy maker organizations like FDA, Medicines & Healthcare Regulatory Agency (MHRA), WHO, European Union (EUDRALEX)-UK, approved all the guidelines of cGMP. Recently GMP is prescribed as "cGMP". The "c" stands for "current" as a reminder that all the techniques, systems, all the processes must be kept up-to-date to consent to the most recent regulations. [42]. cGMP guidelines ensure excellent quality at each step of work. It acts as a safeguard for the quality, purity, strength and identity of cells and related products. This guideline helps to construct a consolidated quality control and management system, helps to hold raw materials quality, helps to develop good SOPs, helps to a build a system of product quality investigation and deviation measurement, and take part in the building of reference laboratories for testing. The necessity of cGMPs are always keep in mind because it is very much important to establish QC. Numerous pharmaceutical companies are executing modern and comprehensive quality control systems and risk management system as a result they hold their quality above the average standards [43]. Quality control (QC) is employed in the GMP framework. Using a broad array of analytical techniques, the QA (Quality assurance) team will identify and quantify all the factors deemed critical for any specific product. Biological products require extensive analysis for characterization. There are some cations related to QC [44] such as-

- identity (confirms correct material);
- quantity (confirms dose parameter);
- purity (confirms material has correct purity);
- impurity (confirms product safety);
- potency (confirms activity);
- sterility including adventitious agents (confirms material is sterile).

9. Overall challenges: cell culture

Culturing techniques for CEF cells or BHK-21 or others is always challenging. The followings are the most important points by maintaining these points researchers or laboratory personnel easily can overcome any possible challenges. The points that need to pay attention such as a). Lab requirement is in the first, set up all the required

materials, chemicals, instruments before the work or research b). Novice personnel should be avoided c). Skilled manpower and staff should be appointed for the work and as a trainer for new one d). Interpretation of work daily e). Establish a strong QC department f). Store all the true data and metadata because it is important to identify problems and also help to design a research g). Calibration of pH meter regular basis is mandatory and pH level follow up because cell health depends on it h). Need to take necessary precautions during culture media preparation i). Maintain and check the required CO_2 percentage based on cell j). Incubation time and temperature vary from cell to cell and it may be vary based on research work k). Transportation process, medium, time, precautions should be describing in transportation SOP l). Pay attention during thawing of the cell because it causes stress on the cell resulting cell might be death m). More careful and be cautious during preservation of cells, changing of media, passaging, trypsinization, harvesting of cells, mycoplasma contamination, and antibiotics treatment (for bacterial contamination) because these are the fundamental steps in cell culture technology. A non-technical or unskilled or novice personnel can easily ruin all the effective work at a time during the above points. That's why need to be more cautious about this n). Certificate of Analysis (COA) is essential for all the work like media preparation, pH level check, cell morphology, cell count, splitting, transportation, cell purchase or sell, and so on. It reduces the risk O). Based on international reference lab or manual SOP should be developed, SOP of how to prepare a good SOP and working guideline can minimize problems and it will take part to handle challenges. Current Good Manufacturing Practice (cGMP), Documentation of lab work, Training facility both for researchers and staff, Validation of work, calibration of instruments regularly can eradicate the problems and smoothly handle all the challenges.

10. Effectiveness of maintaining logbook, training and SOPs

Knowing the difference between a laboratory logbook and a laboratory notebook is very much effective to proceed with laboratory work. Logbook keeps details, usually in a tabulated format on handling and use of equipment, users, time of use, the purpose of use, and watched comments in case any. Other than, the research facility notebook or diary contains whole experimental details (for investigation and analysis), all the readings, results of any calculation, eventually all the supplementary data (graphs, spectra, or chromatograms) preserved in note book. Every research facility action related to the utilization of testing equipment, laboratory environmental records (temperature, humidity, atmospheric pressure, and exposure to light), weighing balance logs (analytical balance is the foremost used device in any research facility), material consumption, chemicals, details of suppliers and supplies are conserved as a proof record. Depending on the work nature vary the types of logbooks. Both notebooks and logs play a vital role in decision making or identify the problems or take part to fix the false result. During an audit, a logbook helps to convince the auditor because it provides evidence-based data. Periodically maintenance, operated by trained staff, calibration, and servicing of instruments increase the shelf life of the instrument, and these logs are stored as a proof document for safe work. Standard operating procedures (SOPs) based work has the same impact on research. On the other hand, training is significant for the advancement of the skills and knowledge of laboratory staff. In a training log the topic of training, date and time, name of trainer and trainees, effectiveness, and also the performance of individuals is reserved [34, 45]. Does every detail clarify in SOP like how to operate an instrument, test, or a machine? How will be eligible to perform? How to monitor (lab,

work, test, etc.) and keep the record? How to calibrate and when? Who certifies and how? How to use chemicals? In a word SOP is fundamental. It is an ideal practice to have procedures for maintaining and controlling laboratory stocks which are clearly defined in specific SOP.

11. Conclusion

In recent years' importance and the application of cell culture cannot explain in a word. Cell culture technology is an extensively accepted technique in the field of molecular biology study and life science research. Due to high feasibility, cell culture practices highly demandable in biopharmaceutical works. This document summarizes the theoretical background, basic concepts regarding cell culture (animal cells, BHK-21, CEF cell culture). The above discussion gives an ideal concept to understand the whole process of cell culture technique, its applications and it helps to build constructive problem-solving confidence related to cell culture along with inspiration. Conclusively, it is said that all the steps regarding cell culture give a fundamental idea of cell culture.

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Conflict of interest

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Abbreviations

ATCC	American Type Culture Collection
BHK	Baby Hamster Kidney
BME	Basal Medium
BOD	Biological Oxygen Demand
BSC	Biosafety Cabinet
BSL	Biosafety Level
CCF	Cell Culture Flask
$CCID_{50}$	Cell Culture Infectious Dose 50
CDC	Center for Disease Control and Prevention
CEF	Chicken Embryo Fibroblast
cGMP	Current Good Manufacturing Practice
CHO	Chinese Hamster Ovary

COA	Certificate of Analysis
CPAs	Cryoprotective Agents
DAPI	4′,6-diamidino-2-phenylindole
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulphoxide
DT	Doubling Time (Population)
EC_{50}	Effective Concentration
EDTA	Ethylenediaminetetraacetic Acid
EID_{50}	Embryo Infective Dosage
ELD_{50}	Embryo Lethal Dosage
ELISA	Enzyme-Linked Immunosorbent Assay
EMEM	Eagle's Minimum Essential Medium
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FFA	Focus forming Assay
FMD	Foot and Mouth Disease
GCCP	Good Cell Culture Practice
GM	Growth Media
GMP	Good Manufacturing Practice
HA	Hemagglutination Assay
hpi	hours post-inoculation
IC_{50}	Concentration of an Inhibitor
IMDM	Iscove's Modified Dulbecco's Medium
IPA	Isopropyl Alcohol
ISO	International Organization for Standardization
LD_{50}	Lethal Dose 50
MHRA	Medicines & Healthcare Regulatory Agency
MOI	Multiplicity of Infection
NIH	National Institute of Health, USA
OECD TG	Organization for Economic Co-Operation and Development Test Guide Line
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PDL	Population Doubling Level
PFU	Plaque-Forming Unit
PPE	Personal Protective Equipment
QA	Quality Assurance
QC	Quality Control
R&D	Research and Development
rDNA	Recombinant Deoxyribonucleic Acid
RDS	Room Data Sheet
ROS	Reactive Oxygen Species
rpm	Rotation Per Minute
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SOP	Standard Operating Procedures
SPF	Specific Pathogen Free
$TCID_{50}$	Tissue Culture Infectious Dose 50
TEM	Transmission Electron Microscopy
UPS	Uninterrupted Power Supply
WHO	World Health Organization

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