BASIC CELL CULTURE TECHNIQUES MANUAL

Quality Operations Laboratory,
University of Veterinary and Animal Sciences, Lahore

Nexus Academic Publishers (NAP)
Lahore Pakistan

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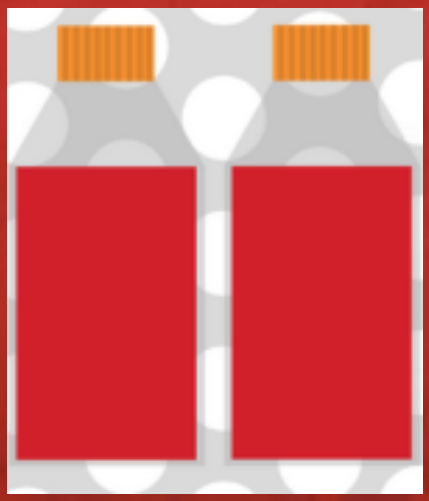

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DEDICATIONS

TO ALL STUDENTS

First Published in Pakistan in June 2014

The first edition published in 2014 by

Nexus Academic Publishers (NAP)

1–B Opposite School –4, Gate # 2, Old Officer Colony Zarar Shaheed Road, Sadar Lahore Cantt. Lahore, Pakistan Phone: 0092 322 4742353

email: info@nexusacademicpublishers.com

Nexus Academic Library Cataloguing in Publication Data

A catalogue record for this book is available for the Nexus Academic Publishers

Typeset in 8.5 Californian FB by Nexus Academic Publishers, Punjab, Lahore Cantt (Pakistan)

Printed and bound in Pakistan

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Acknowledgement

I would like to thank my colleagues for their support during preparation of this manual. I would like to thank Prof. Dr. Tahir Yaqub for his kind encouragement and appreciation.

Contents

CHAPTER

1 Introduction of Cell Culture

Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favorable artificial environment. The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been established. Tissue culture as a technique was first used almost 100 years ago to elucidate some of the most basic questions in developmental biology. Ross Harrison at the Rockefeller Institute, in an attempt to observe living, developing nerve fibers, cultured frog embryo tissues in plasma clots for 1 to 4 weeks (Harrison, 1907). He was able to observe the development and outgrowth of nerve fibers in these cultures. In 1912, Alexis Carrel, also at the Rockefeller Institute, attempted to improve the state of the art of animal cell culture with experiments on the culture of chick embryo tissue: Carrel succeeded in expanding the possibilities of cell culture by keeping fragments of chick embryo heart alive and beating into the third month of culture and growing chick embryo connective tissue for over 3 months. Although animal cell culture was first successfully under taken by Ross Harrison, it was not until the late 1940's to early 1950's that several developments occurred that made cell culture widely available as a tool for scientists (Stoppini.et al,. 1991).

At that time the next focus was to grow human cells in the laboratory, it's very hard to grow most human cells in the lab for an extended period. In fact, once removed from the human body, most cells will either die immediately or reproduce only a limited number of times. That's why it was so significant in 1951 that this barrier was overcome for the first time, using cancer cells taken from a 31 year old African American woman named Henrietta Lacks (Masters, 2002; Banker and Cowan, 1977). HeLa became the human cell line for all kinds of scientific inquiry. While other immortalized lines are now available, HeLa remains the most widely used cell line in biomedical research. HeLa cells have also served as the foundation for developing modern vaccines, including the polio vaccine; understanding viruses and other infectious agents; and devising new medical techniques, such as in vitro fertilization (Francis, 2013).

Primary Culture

Primary culture refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate i.e., confluence. At this stage, the cells have to be sub cultured i.e., passaged by transferring them to a new vessel with fresh growth medium to provide more room for continued growth (Abbott, 2003).

Secondary Culture

After the first subculture, the primary culture becomes secondary culture also known as cell line. Cell lines derived from primary cultures have a limited life span i.e., they are finite and as they are passaged, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population.

Cell Strain

If a subpopulation of a cell line is positively selected from the culture by cloning or some other method, this cell line becomes a cell strain. A cell strain often acquires additional genetic changes subsequent to the initiation of the parent line.

CHAPTER 2 Material and Methods

Cell Culture Equipment

The specific requirements of a cell culture laboratory depend mainly on the type of research conducted; for example, the needs of mammalian cell culture laboratory specializing in cancer research is quite different from that of an insect cell culture laboratory that focuses on protein expression. However, all cell culture laboratories have the common requirement of being free from pathogenic microorganisms (i.e., asepsis), and share some of the same basic equipment that is essential for culturing cells.

This section enlists the equipment common to most cell culture laboratories as well as beneficial equipment that allow the work to be performed more efficiently or accurately or permits wider range of assays and analyses.

Basic Equipment

- Biosafety–cabinet.
- Incubator humid CO_2 incubator recommended.
- Water bath
- Centrifuge
- Refrigerator and freezer (–20°C)
- Cell counter e.g., Countess Automated Cell Counter or hemocytometer.
- Inverted microscope
- Liquid nitrogen (N_2) freezer or cryo–storage container
- Autoclave.

Expanded Equipment

- Aspiration pump (peristaltic or vacuum)
- pH meter
- Confocal microscope
- Flow cytometer

Additional Supplies

- Cell culture vessels (e.g., flasks, Petri dishes, roller bottles, multi–well plates)
- Pipettes and pipettors
- Syringes and needles
- Waste containers
- Media, sera and reagents.

Cell Culture Laboratory

Aseptic Work Area

The major requirement of a cell culture laboratory is the need to maintain an aseptic work area that is restricted to cell culture work. Although a separate tissue culture room is preferred, a designated cell culture area within a larger laboratory can still be used for sterile handling, incubation, and storage of cell cultures, reagents, and media. The simplest and most economical way to provide an aseptic condition is to use a cell culture hood i.e., biosafety cabinet.

Cell Culture Hood

The cell culture hood provides an aseptic work area while allowing the containment of infectious splashes or aerosols generated by many microbiological procedures. Three kinds of cell culture hoods, designated as Class I, II and III, have been developed to meet varying research and clinical needs.

Classes of Cell Culture Hoods

- Class I cell culture hoods offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological

techniques, but they do not provide cultures protection from contamination. They are similar in design and air flow characteristics to chemical fume hoods.

- Class II cell culture hoods are designed for work involving BSL–1, 2, and 3 materials, and they also provide an aseptic environment necessary for cell culture experiments. A Class II biosafety cabinet should be used for handling potentially hazardous materials e.g., primate–derived cultures, virally infected cultures, Radio–isotopes, carcinogenic or toxic reagents.
- Class III biosafety cabinets are gas–tight, and they provide the highest attainable level of protection to personnel and the environment. A Class III biosafety cabinet is required for work involving known human pathogens and other BSL–4 materials.

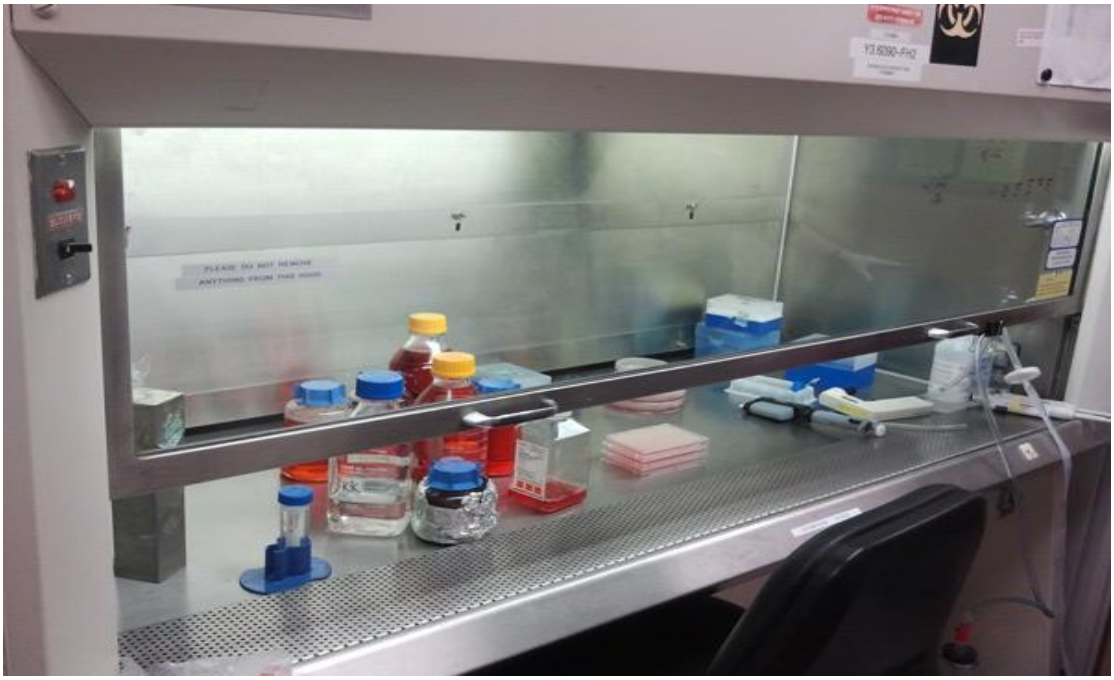


Figure 1: Cell Culture Hood

Air Flow Characteristics of Cell Culture Hoods

Cell culture hoods protect the working environment from dust and other air born contaminants by maintaining a constant, unidirectional flow of HEPA–filtered air over the work area. The flow can be horizontal, blowing parallel to the work surface, or it can be vertical, blowing from the top of the cabinet onto the work surface.

Depending on its design, a horizontal flow hood provides protection to the culture (if the air flowing towards the user) or to the user (if the air is drawn in through the front of the cabinet by negative air pressure inside).

Vertical flow hoods, on the other hand, provide significant protection to the user and the cell culture (Banker and Cowan, 1977).

Clean Benches

Horizontal laminar flow or vertical laminar flow "clean benches" are not Bio safety cabinets; these pieces of equipment discharge HEPA–filtered air from the back of the cabinet across the work surface toward the user, and they may expose the user to potentially hazardous materials. These devices only provide product protection. Clean benches can be used for certain clean activities, such as the dust–free assembly of sterile equipment or electronic devices, and they should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials.

Cell Culture Hood Layout

A cell culture hood should be large enough to be used by one person at a time, be easily cleanable inside and

outside, have adequate lighting, and be comfortable to use without requiring awkward positions. Disinfect each item placed in the cell culture hood by spraying them with 70% ethanol and wiping clean. The arrangement of items within the cell culture hood usually adheres to the following right–handed convention, which can be modified to include additional items used in specific applications.

- A wide, clear work space in the center with your cell culture vessels
- Pipettor in the front right, where it can be reached easily
- Reagents and media in the rear right to allow easy pipetting
- Tube rack in the rear middle holding additional reagents
- Small container in the rear left to hold liquid waste.

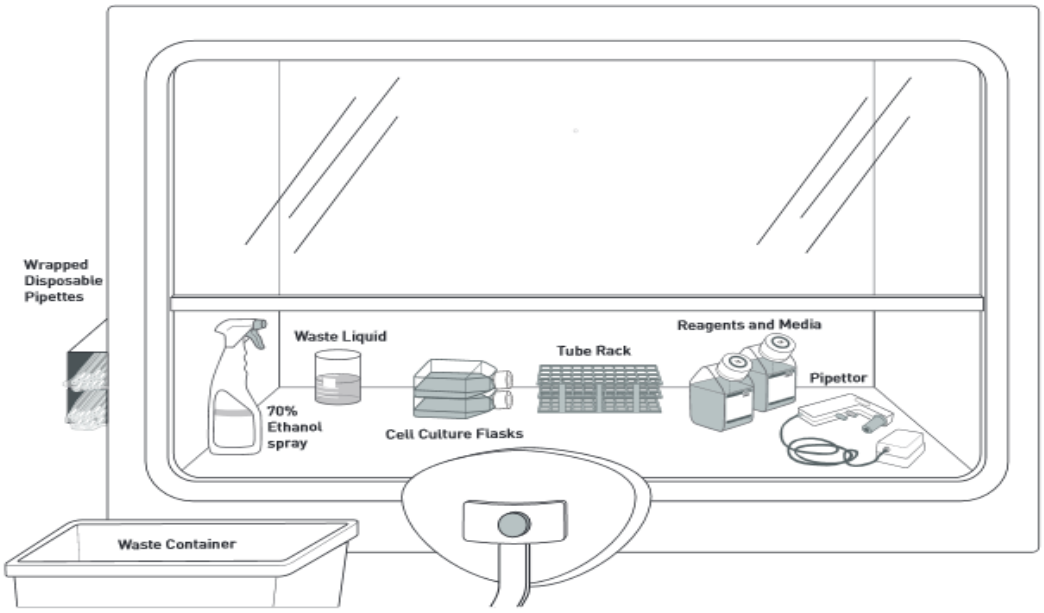


Figure 2: The basic layout of a cell culture hood for right–handed workers. Left–handed workers may switch the positions of the items laid out on the work surface

Incubator

Main reason of using incubator is to provide cells with appropriate environment. Incubators with stainless steel make it easy to wash and make corrosion free. Forced air circulation and ±0.2°C temperature control are basic necessity for good incubators.

Types of Incubators

There are two basic types of incubators,

1. Dry incubators and
2. Humid CO_2 incubators.

Dry Incubators

It is economical, but requires that the cell culture to be incubated in sealed flasks to prevent evaporation. Placing a water dish in a dry incubator can provide some humidity, but they do not allow precise control of atmospheric conditions in the incubator.

Humid CO_2 Incubators

It is expensive, but allows superior control of culture condition. They can be used to incubate cells cultured in Petri dishes or multi–well plates, which require a controlled atmosphere of high humidity and increased CO_2 tension.

Storage

A cell culture laboratory should have storage areas for liquids such as media and reagents, for chemicals such as drugs and antibiotics, for consumables such as

disposable pipettes, culture vessels, and gloves, for glassware such as media bottles and glass pipettes, for specialized equipment, and for tissues and cells. Glassware, plastics, and specialized equipment can be stored at ambient temperature on shelves and in drawers; however, it is important to store all media, reagents, and chemicals according to the instructions on the label. Some media, reagents and chemicals are sensitive to light while their normal laboratory use under lighted conditions is tolerated, they should be stored in the dark or wrapped in aluminum foil when not in use (Ryan, 2014)

Figure 3: CO_2 incubator

Refrigerators

For small cell culture laboratories, a domestic refrigerator preferably one without an auto defrost freezer is an adequate and inexpensive piece of equipment for storing reagents and media at 2–8°C. For larger laboratories, a cold room restricted to cell culture is more appropriate. Make sure that the refrigerator or the cold room is cleaned regularly to avoid contamination (Hadeler, 1995)

Freezers

Most cell culture reagents can be stored at –5°C to –20°C; therefore an ultra–deep freezer i.e., a –80°C freezer is optional for storing most reagents. A domestic freezer is a cheaper alternative to a laboratory freezer. While most reagents can withstand temperature oscillations in an auto defrost i.e., self–thawing freezer, some reagents such as antibiotics and enzymes should be stored in a freezer that does not auto defrost. (Rae, 1997)

Cryogenic Storage

Cell lines in continuous culture are likely to suffer from genetic instability as their passage number increases; therefore, it is essential to prepare working stocks of the cells and preserve them in cryogenic storage. Do not store cells in –20°C or –80°C freezers, because their viability quickly decreases when they are stored at these temperatures. There are two main types of liquid–nitrogen storage systems, vapor phase and liquid phase, which come as wide–necked or narrow–necked storage containers (Mitalipova, 2005). Vapor phase systems minimize the risk of explosion with cryo storage tubes, and are required for storing bio hazardous materials, while the liquid phase systems usually have longer static holding times, and are therefore more economical. Narrow–necked containers have a slower nitrogen evaporation rate and are more economical, but wide–necked containers allow easier access and have a larger storage capacity.

Figure 4: layout of a typical cell culture laboratory

Media

Chemically defined basal liquid media are used to provide nutrients for cell growth in research, diagnostic and manufacturing applications. Typical cell culture

media contain a mixture of defined nutrients dissolved in a buffered physiological saline solution. Most media contain salts, amino acids, sugar, vitamins and other organic nutrients.

The selection of a basal medium for cell culture applications is primarily dependent on the chemical definition of the basal medium, the type of cell to be grown, and the culture system being employed (Schlegel, 1993)

Basic Constituents of Media

- Inorganic salts
- Carbohydrates
- Amino Acids
- Vitamins
- Fatty acids and lipids
- Proteins and peptides
- Serum
- Trace Elements

Each type of constituent performs a specific function as outlined below:

Inorganic Salts

The inclusion of inorganic salts in media performs several functions. Primarily they help to retain the osmotic balance of the cells and help regulate membrane potential by provision of sodium, potassium and calcium ions. All of these are required in the cell matrix for cell attachment and as enzyme cofactors.

Buffering Systems

Most cells require pH conditions in the range 7.2–7.4 and close control of pH is essential for optimum culture conditions. There are major variations to this optimum. Fibroblasts prefer a higher pH (7.4–7.7) where as, continuous transformed cell lines require more acid conditions pH (7.0–7.4) (Pollard and Walker, 1990)

Regulation of pH is particularly important immediately following cell seeding when a new culture is establishing and is usually achieved by one of two buffering systems;

(i) a "natural" buffering system where gaseous CO_2 balances with the CO_3/HCO_3 content of the culture medium and

(ii) Chemical buffering using a zwitter ion called HEPES.

HEPES has superior buffering capacity in the pH range 7.2–7.4 but is relatively expensive and can be toxic to some cell types at higher concentrations. HEPES buffered cultures do not require a controlled gaseous atmosphere (Ozturk and Hu, 2005)

Most commercial culture media include phenol red as a pH indicator so that the pH status of the medium is constantly indicated by the color. Usually the culture medium should be change/replenish if the color turns yellow (acid) or purple (alkali).

Carbohydrates

The main source of energy is derived from carbohydrates generally in the form of sugars. The major sugars used are glucose and galactose, however, some media contain maltose or fructose. The concentration of sugar varies from basal media containing 1g/L to 4.5g/L in some more complex media. Media containing the higher concentration of sugars are able to support the growth of a wider range of cell types (Singh *et al*, 2013).

Amino Acid

Amino acids are the building blocks of proteins. 'Essential' amino acids must be added to culture media as cells are not able to synthesize these themselves. The concentration of amino acids in the culture medium will determine the maximum cell density that can be achieved, once depleted the cells will no longer be able to proliferate. In relation to cell culture, glutamine, an essential amino acid, is particularly significant (Dichter, 1978).

Vitamins

Serum is an important source of vitamins in cell culture. However, many media are also enriched with vitamins making them consistently more suitable for a wider range of cell lines. Vitamins are precursors for numerous co–factors. Many vitamins, especially B group vitamins, are necessary for cell growth and proliferation and for some lines the presence of B12 is essential. Some media also have increased levels of vitamins A and E. The vitamins commonly used in media include riboflavin, thiamine and biotin. (Washington, 1996)

Proteins and Peptides

These are particularly important in serum free media. The most common proteins and peptides include albumin, transferrin, fibronectin and fetuin and are used

to replace those normally present through the addition of serum to the medium.

Fatty Acids and Lipids

Like proteins and peptides these are important in serum free media since they are normally present in serum e.g. cholesterol and steroids essential for specialized cells.

Trace Elements

These include trace elements such as zinc, copper, selenium and tri–carboxylic acid intermediates. Selenium is a detoxifier and helps remove oxygen free radicals (Schlegel, 1993)

Serum

Serum is a complex mix of albumins, growth factors and growth inhibitors and is probably one of the most important components of cell culture medium. The most commonly used serum is fetal bovine serum (FBS). Other types of serum are available including newborn calf serum and horse serum (Parija, 2009). The quality, type and concentration of serum can all affect the growth of cells and it is therefore important to screen batches of serum for their ability to support the growth of cells.

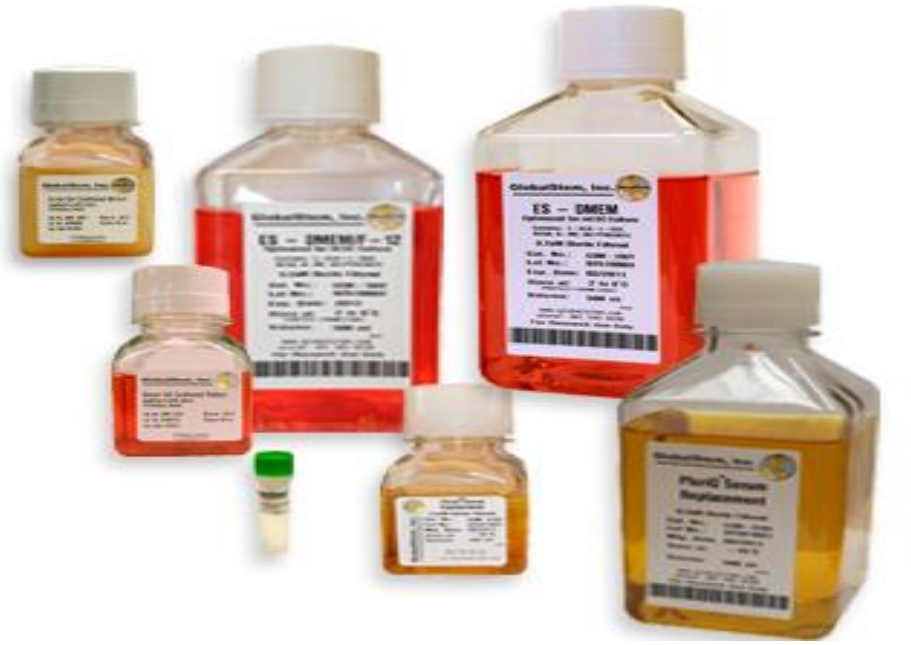


Figure 4: different media used to culture cells

List of Different Media Used in Cell Culturing

1. BME – Basal Medium Eagle
2. DMEM – Dulbecco's Modified Eagle Medium
3. DMEM / F12 Media
4. F–10 Nutrient Mixture
5. F–12 Nutrient Mixture
6. GMEM – Glasgow Modified Minimum Essential Medium
7. IMDM – Iscove's Modified Dulbecco's Medium
8. Leibovitz's L–15 Medium
9. McCoy's 5A Medium
10. MCDB 153 (Modified)
11. Media 199
12. MEM – Minimal Essential Medium
13. MEMA – Minimum Essential Media Alpha
14. RPMI 1640 Medium(Davis, 2011)

CHAPTER

3 Techniques in Cell Culture

Collection of Cells

- First of all choose any organism as source of cell then in presence of surgeons and expert technicians, biopsy of organism is done.
- Liver cells, blood cell, bone marrow cells or any other cells are obtained by cutting that specific organ having these cells.
- Place them in a container having antibiotics and label the container.
- Place the container in freezer but do not freeze the cell samples.
- Don't let the container to get warm and use picnic chillers or small fridge to transport at a distance.
- Do not carry more than one cell types as chances of cross contamination increases.
- After collection first step is disaggregation in cell culture lab (Baron.1996).

Primary Culturing

- Primary culture refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate i.e., confluence. At this stage, the cells have to be sub cultured i.e., passaged by transferring them to a new vessel with fresh growth medium to provide more room for continued growth (Abbott, 2003).
- The first step in isolating cells of a uniform type from a tissue that contains a mixture of cell types is to disrupt the extracellular matrix that holds the cells together. The best yields of viable dissociated cells are usually obtained from fetal or neonatal tissues.
- The tissue sample is typically treated with proteolytic enzymes such as trypsin and collagenase to digest proteins in the extracellular matrix and with agents ethylene diamine tetraacetic acid (EDTA) that bind, or chelate, the Ca^{2+} on which cell–cell adhesion depends.
- The tissue can then be tearing apart into single living cells by gentle agitation.
- Several approaches are used to separate the different cell types from a mixed cell suspension. One exploits differences in physical properties. Large cells can be separated from small cells and dense cells from light cells by centrifugation.
- Another approach is based on the tendency of some cell types to adhere strongly to glass or plastic, which allows them to be separated from cells that adhere less strongly (Cooper and Hausman, 2000).
- An important refinement of this last technique depends on the specific binding properties of antibodies.
- Antibodies that bind specifically to the surface of only one cell type in a tissue can be coupled to various matrices such as collagen, polysaccharide beads, or plastic to form an affinity surface to which only cells recognized by the antibodies can adhere.
- The bound cells are then recovered by gentle shaking, by treatment with trypsin to digest the

proteins that mediate the adhesion, or, in the case of a digestible matrix such as collagen, by degrading the matrix itself with enzymes such as collagenase. (Bruce *et al*., 2002).

- The tissue will go through stages of rinsing, dissection, and either mechanical disaggregation or enzymatic digestion in trypsin and/or collagenase.
- It is often desirable not to have a complete single–cell suspension, and many primary cells survive better in small clusters.
- Disaggregated tissue will contain a variety of different cell types, eliminate those that are not required. (Saalbach *et al*, 1997).
- The cell population can then be further enriched by selection of the correct medium, many of which are now available commercially and supplementing this with growth factors.
- Survival and enrichment may be improved in some cases by coating the substrate with gelatin, collagen, laminin, or fibronectin (Freshney, 2005).

Sub Culturing

- Frequently, the number of cells obtained at primary culture may be insufficient to create constructs suitable for grafting. Sub–culturing gives the opportunity to expand the cell population, apply further selective pressure with a selective medium, and achieved a higher growth fraction and allows the generation of replicate cultures for characterization, preservation by freezing, and experimentation.
- Briefly, subculture involves the dissociation of the cells from each other and the substrate to generate single–cell suspension that can be quantified.
- Reseeding this cell suspension at a reduced concentration into a flask or dish generates a secondary culture, which can be grown up and sub cultured again to give a tertiary culture, and so on (Dallman and Lamb, 2000).
- In most cases, cultures dedifferentiate during serial passaging but can be induced to re–differentiate by cultivation on a 3D scaffold in the presence of tissue–specific differentiation factors e.g., growth factors and physical stimuli.
- Cell's ability to de–differentiate decreases with passaging. It is thus essential to determine, for each cell type, source, and application, a suitable number of passages during subculture. (Freshney, 2005).

Viral Infection and Harvesting

- Virus is inoculated in flask having adapted cells and then media is exchanged and poured in different flasks incubate at 37 °C for 24 hours.
- Virus should be filtered with 0.2µm micro filter. Cells are harvested when the cells have reached a population density which suppresses growth. Ideally, cells are harvested when they are in a semi–confluent state and are still in log phase.
- Cells that are not passaged and are allowed to grow to a confluent state can sometime lag for a long period of time and some may never recover.
- It is also essential to keep your cells as happy as possible to maximize the efficiency of transformation. Most cells are passaged (or at least fed) three times a week (Devis, 2011).
    - Suspension culture. Suspension cultures are fed by dilution into fresh medium.
    - Adherent cultures. Adherent cultures that do not need to be divided can simply be fed by removing the old medium and replacing it with fresh medium.
- When the cells become semi–confluent, several methods are used to remove the cells from the growing surface so that they can be diluted.
- **Mechanical:** A rubber spatula can be used to physically remove the cells from the growth surface. This method is quick and easy but is also disruptive to the cells and may result in significant cell death. This method is best when harvesting many different samples of cells for preparing extracts, i.e., when viability is not important.
- **Proteolytic enzymes:** Trypsin, collagenase, or proteinase, usually in combination with EDTA, causes cells to detach from the growth surface. This method is fast and reliable but can damage the cell surface by digesting exposed cell surface proteins. The proteolysis reaction can be quickly terminated by the addition of complete medium containing serum
- **EDTA:** alone can also be used to detach cells and seems to be gentler on the cells than trypsin. The standard procedure for detaching adherent cells is as follows:

1. Visually inspect daily
2. Release cells from monolayer surface

- Wash once with a buffer solution
- Treat with dissociating agenYS
- Observe cells under the microscope. Incubate until cells become rounded and loosen when flask is gently tapped with the side of the hand.
- Transfer cells to a culture tube and dilute with medium containing serum.
- Spin down cells, remove supernatant and replace with fresh medium.
- Count the cells in a hemacytometer, and dilute as appropriate into fresh medium.

Cryopreservation

- If a cell line can be expanded sufficiently, preservation of cells by freezing will allow secure stocks to be maintained without aging and protect them from problems of contamination, incubator failure, or medium and serum crises.
- Ideally, 1×10^6 –1×10^7 cells should be frozen in 10 ampoules, but smaller stocks can be used if a surplus is not available.
- The normal procedure is to freeze a token stock of one to three ampoules as soon as surplus cells are available, then to expand remaining cultures to confirm the identity of the cells and absence of contamination, and freeze down a seed stock of 10–20 ampoules.
- One ampoule, thawed from this stock, can then be used to generate a using stock.
- In many cases, there may not be sufficient doublings available to expand the stock as much as this, but it is worth saving some as frozen stock, no matter how little, although survival will tend to decrease below 1×10^6 cells/ml and may not be possible below 1×105 cells/ml (Freshney, 2005).

CHAPTER

4 Culturing BHK and Vero Cell Lines

BHK 21 cells and Vero cells were the most commonly used cell for vaccine production.

Collection and Storage

Vero and BHK cells were collected by the courtesy of ATCC the culture bank of cells. Cells were shipped on dry ice. Cells were stored in liquid nitrogen upon receipt. Cells were processed at normal temperature.

Contents

One vial contained 3 x 10 baby hamster kidney (BHK) cells in 1 ml of a MEM, 10% fetal bovine serum, 10% DMSO. The BHK cell line was derived from baby Syrian hamster (*Mesocricetus auratus*) kidney (Macpherson and Stoker, 1962). The medium for BHK cells was αMEM or DMEM. Complete medium for BHK cells were αMEM + 2 mM L–glutamine + 5% fetal bovine serum. FBS did not need to be heat inactivated to use with BHK cells. 10% FBS helps the cells to grow fast. Cells were grown in a humidified, 37°C, 5% CO_2 incubator. If cells are split at 1:5, it took 1–2 days to reach 80–90% confluence.

The cells have a tendency to clump in complete medium. In general, this is not a problem except when preparing the cells for electroporation. In this case, care must be taken to avoid clumps. PBS is required to keep the cells from clumping during electroporation.

General Cell Handling

All solutions and equipment that come in contact with the cells must be sterile.

Always use proper sterile technique and work must be done in a laminar flow hood. Use cells that are 80–90% confluent and › 90% viability for transfections and infections. Before starting experiments, be sure to have cells established and also have some frozen stocks on hand. For general maintenance of cells, pass BHK cells when they are 80–90% confluent (1–2 days) and split at a 1:5 dilution. For example, transfer 2 ml of a 10 ml cell suspension (without trypsin/EDTA) to a new 75 cm^2 flask and add 10 ml fresh medium. Thawed cells should be 80 to 85% viable and healthy; log phase cultures should be › 90% viable.

Culturing BHK Cells

Make sure to have the following solutions and supplies available

- 15 ml sterile, conical tubes
- 5, 10, and 25 ml sterile pipettes
- Cryovials
- PBS
- Hemacytometer
- α–MEM medium
- Tissue culture grade 200 mM L–glutamine
- FBS
- Complete αMEM medium (αMEM + 2 mM L–glutamine + 5% FBS,)
- Freezing Medium (αMEM medium + 2 mM L–glutamine + 10% FBS + 10% DMSO,
- Table–top centrifuge
- 75 cm^2flasks, 175 cm^2 flasks and 35 mm plates (other flasks and plates may be used)
- Trypsin or (EDTA) solution or other trypsin solution.

Passaging of BHK Cells

1. When cells are ~80–90% confluent, remove all medium from the flask.
2. Washed cells once with 10 ml PBS to remove medium. Serum contains inhibitors of trypsin.
3. Add 5 ml of trypsin or EDTA solution to the monolayer and incubated 1 to 5minutes at room temperature until cells detached. Checked the cells under a microscope and confirmed that most of the cells had detached. If cells were still attached,

incubate a little longer until most of the cells have detached.

4. Once the cells have detached, briefly pipet the solution up and down to break up clumps of cells.
5. Add 5 ml of complete αMEM to stop trypsinization. Centrifuge cells at 250 x g for 5 minutes.
6. Aspirate the supernatant and resuspend the cells in 10 ml complete αMEM.
7. To maintain cells in 75 cm^2 flasks, transfer 2 ml of the 10 ml cell suspension from Step 6 to a new 75 cm^2 flask and add 10 ml fresh, complete αMEM medium.
8. To expand cells, transfer 3 to 4 ml of the cell suspension to a 175 cm^2 flask (3 flasks total) and add fresh, complete αMEM medium to a final volume of 30 ml.
9. Incubate flasks in a humidified, 37°C, 5% CO_2 incubator.

Complete MEM Medium

To 500 ml αMEM add 5% FBS and 5 ml of 200 mM L–glutamine solution

Freezing Medium

- ✓ αMEM containing 2 mM L–glutamine,
- ✓ 10% FBS, and
- ✓ 10% DMSO.

Phosphate Buffered Saline

It is used for the washing cells only. The solution did not need to be RNase–free.

- ✓ 137 mM NaCl
- ✓ 2.7 mM KCl
- ✓ 10 mM Na_2HPO_4
- ✓ 1.8 mM KH_2PO_4

1. Dissolve: 8 g NaCl 0.2 g KCl 1.44 g Na_2HPO_4 0.24 g KH_2PO_4 in 800 ml deionized water.
2. Adjust pH to 7.4 with concentrated HCl.
3. Bring the volume to 1 liter and autoclave for 20 minutes on liquid cycle.
4. Store at +4°C or room temperature.

Vero Cell Culture

The Vero cell line was derived from kidney epithelial cells of the African Green Monkey. Vero cells are frequently used in large–scale vaccine production because they grow well in bioreactors with a high viral yield.

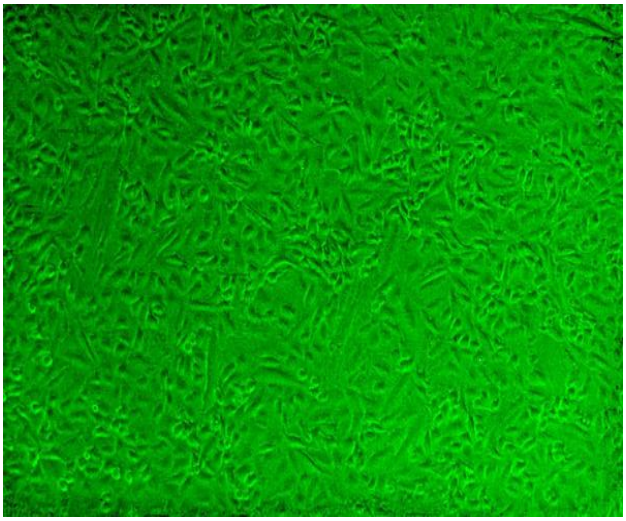
Traditionally, Vero cell culture media contain fetal bovine serum or other animal–derived media supplements. The use of these animal products creates many challenges, primarily safety concerns. Animal–derived supplements can infect cells with bacteria, viruses, and prions, particularly bovine spongiform encephalopathy (BSE or mad cow disease). In addition, productivity suffers when animal–derived supplements are used due to the high level of variability and lot–to–lot inconsistencies in these products and virus production titers and viral yields can vary significantly between batches. Poor Vero cell viability leads to longer cell doubling times and long production cycle times.

Complete Growth Medium

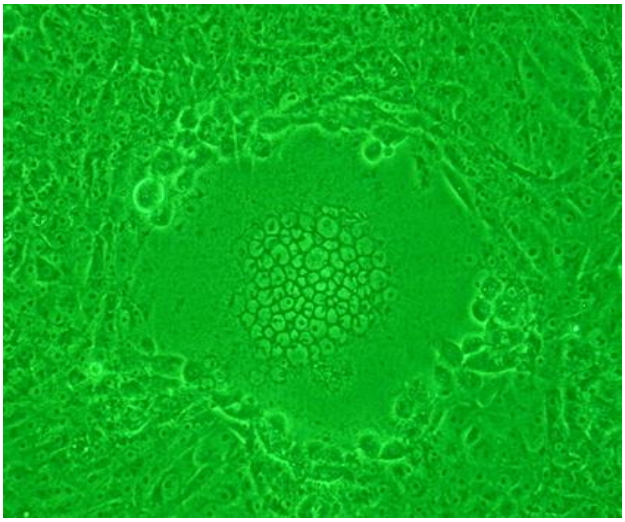
The base medium for this cell line was Eagle's Minimum Essential Medium. To make the complete growth medium, following components are added to the base medium: fetal bovine serum to a final concentration of 10%.

Sub culturing

1. Volumes were given for a 75 cm^2 flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.
2. Remove and discard culture medium.
3. The cell layer should be rinsed with 0.25% (w/v) Trypsin 0.53 mM EDTA to remove all traces of serum that contains trypsin inhibitor.
4. Then add 2.0 to 3.0 mL of Trypsin–EDTA solution to flask and observed cells under an inverted microscope until cell layer was dispersed (usually within 5 to 15 minutes).
5. To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach.
6. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
7. Add 6.0 to 8.0 mL of complete growth medium and aspirated cells by gently pipetting.
8. Add appropriate aliquots of the cell suspension to new culture vessels.
9. Incubate cultures at 37°C.
10. Subcultivation Ratio: A sub cultivation ratio of 1:3 to 1:6 is recommended Medium Renewal: 2 to 3 times per week.



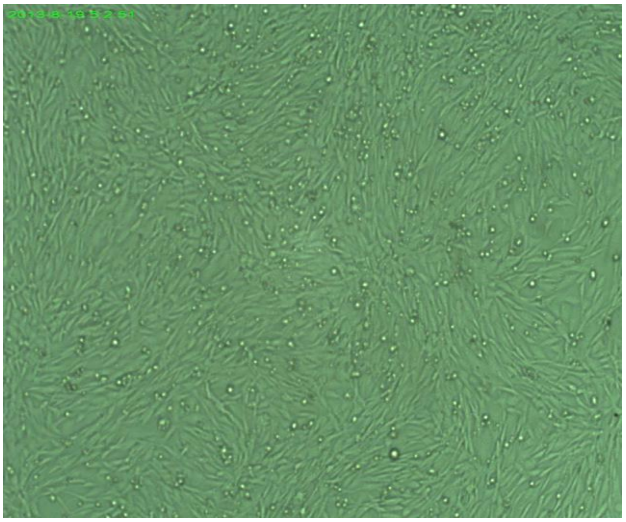
A



B

Figure 1: (A) it shows the 90% confluent Vero cells.(B) when Vero cells are infected with PPR virus a characteristic syncytium is formed, cells lost their confluence and cytopathic effect of virus can be seen in the figure

BHK Cells



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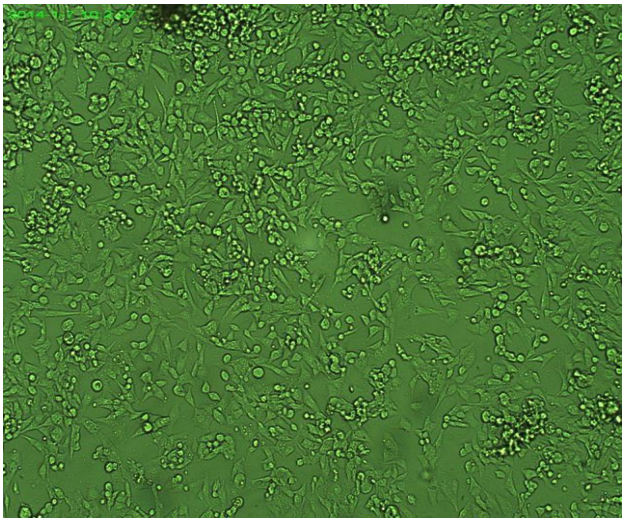

B

Figure 2: (A) Shows the confluent BHK Cells. (B) The results of CPEs of FMD on BHK cells, BHK cells get rounded and detach from the flask

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