

## Review

## Basics of animal cell culture: Foundation for modern science

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Received 29 June, 2016; Accepted 5 August, 2016

The culture of animal cells is one of the major aspects of science which serves as a foundation for most of our recent discoveries. The major areas of application include cancer research, vaccine manufacturing, recombinant protein production, drug selection and improvement, gene therapy, stem cell biology, monoclonal antibody production, *in vitro* fertilization technology, cryopreservation and *in vitro* production of hormones. Cells can be propagated, expanded and divided into identical replicates, which can be characterized, purified and preserved by freezing. This article reviews the basic aspects of animal cell culture for modern day research.

**Key words:** Animal cell culture, cell freezing, cell preservation.

### INTRODUCTION

The culture of animal cells and tissues is a generally and widely used technique that involves isolation of cells, tissues and organs from animals and growing them in an *in vitro* or artificial environment. The term culture means to keep alive and grow in an appropriate medium that simulates the natural conditions. The list of different cell types which can now be grown in culture include connective tissues such as fibroblasts, skeletal, cardiac and smooth muscle, epithelial tissues, neural cells, endocrine cells and many different types of tumour cells (Merten, 2006).

*In vitro* culture has been proven to be the most valuable method to study the functions and mechanism of operations of many cells. A particular group of cells can be cultured in large quantities to study their cellular

activities, differentiations and proliferations. Cell culture is highly essential to biotechnology; the major areas of application of animal cell culture are; cancer research, vaccine manufacturing, recombinant protein production, drug selection and improvement, gene therapy, stem cell biology and *in vitro* fertilization technology.

Growing tissues of living organisms outside the body are made possible in an appropriate culture medium, which contains a mixture of nutrients either in solid or liquid form. Nutritional factors like serum,  $Ca^{2+}$  ions, hormones etc. can be added to the medium to aid growth, differentiation and proliferation of cells. Cells can be propagated, expanded and divided into identical replicates, which can be characterized and preserved by freezing. They can also be purified phenotypically by

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growth in selective media. Historical landmarks in the development of cell culture are presented in Table 1. The objective of this review was to provide useful and basic information on animal cell culture for early career scientists.

## PROCEDURE OF CELL CULTURE

### Primary culture

Freshly isolated cultures are known as primary cultures until they are passaged or subculture. They are usually heterogeneous, and have a low growth fraction, but they are more representative of the cell types in the tissue from which they were derived and in the expression of tissue specific properties. The first step in obtaining the primary culture is isolation of tissues from the whole part or organ, followed by disaggregation of cells from the tissues. This is done by addition of low trypsin to the tissue for proper disintegration and isolation of cells. Trypsin is added to the tissues in order to degrade extracellular proteases and glycosidase (Huang et al., 2010). The externally exposed proteins are digested by the action of trypsin for dissociation of cells of the tissues in order to harvest individual cells. The cells obtained after trypsin digestion are incubated in the presence or absence of serum and culture in a medium.

### Subculture culture

A subculture is a new culture taken from a primary culture and grown separately in the culture medium. Subculture allows the expansion of the culture (it is now known as a cell line). The advantage of sub-culturing primary culture into a cell line is the provision of large amounts of consistent material suitable for prolonged use (Nguyen et al., 2012). The subculture does not need trypsinization. The medium required is based on the type of specialized cells in culture. This subculture may not need serum for continuous propagation (Baltz and Tartia, 2010).

### Monolayer culture

This is a type of culture in which the bottom of the culture plate is covered by a continuous or a single layer of cells in a culture medium (Hazen et al., 1995). They do not require enzymatic or mechanical dissociation. Growth is limited by concentration of cells in the medium, which allows easy scale-up.

### Suspension culture

Suspension cultures are cells which can be grown within suspension of the medium. They are easier to passage as there is no need to detach them. They do not require

enzymatic or mechanical dissociation.

### Adherent culture

Cells are dissociated enzymatically before they are cultured. They require periodic passaging, but allow easy visual inspection under inverted microscope. They are referred to as anchorage dependent cells.

### Seeding density and cell propagation

The conditions which favour cell proliferation are low cell density, low  $\text{Ca}^{2+}$  concentration (100 to 600uM) and the presence of growth factors such as epidermal growth factor (EGF), fibroblast growth factors (FGF), and platelet derived growth factor (PDGF). High cell density ( $>10^5$  cells/  $\text{cm}^2$ ) will favour cytostasis and differentiation. Different conditions are therefore required for propagation and differentiation, and hence an experimental protocol may require a growth phase to increase cell number, this is followed by a non-growth maturation phase, which allows replication of samples and an increased in the expression of differentiated functions (De Felici et al., 2004).

In general, cultures derived from embryonic tissues will survive and grow better than those of the adult. They presumably reflect the lower level of specialization and presence of replicating precursor or stem cells in the embryo (De Felici et al., 2004). Adult tissues will usually have a lower growth fraction and high proportion of non-replicating specialized cells. Cells cultured from neoplasia, however, can express at least partial differentiation, e.g. B16 Mouse melanoma, while retaining capacity to divide (De Felici et al., 2004; Nguyen et al., 2012). The list of main equipment required for cell culture is provided in Table 3.

## TYPE OF CULTURE SYSTEMS

### Batch

This can be defined as the usual type of culture in which cells are inoculated into a fixed volume of medium. As the culture grows, nutrients are consumed and metabolites are accumulated. The environment is therefore continually changing and this, in turn, enforces changes to cell metabolism, often referred to as physiological differentiation. Batch culture is suitable for both monolayer and suspension cells (Shiloach and Fass, 2005). The media are added to the culture in three different ways:

- (1) By replacing a constant fraction of the culture with an equal volume of fresh medium.
- (2) By increasing continuously the volume at a constant rate, but withdrawing culture aliquots at intervals.

**Table 1.** Historical landmarks in the development of cell culture.

Year	Historical landmarks in the development of cell culture
1878	Claude Bernard proposed that physiological systems of an organism can be maintained in a living system after the death of an organism.
1885	Roux maintained embryonic chick cells in a saline culture.
1897	Loeb demonstrated the survival of cells isolated from blood and connective tissue in serum and plasma.
1903	Jolly observed cell division of salamander leucocytes <i>in vitro</i> .
1907	Harrison cultivated frog nerve cells in a lymph clot held by the 'hanging drop' method and observed the growth of nerve fibers <i>in vitro</i> for several weeks. He was considered by some as the father of cell culture
1910	Burrows succeeded in long term cultivation of chicken embryo cell in plasma clots. He made detailed observation of mitosis.
1911	Lewis and Lewis made the first liquid media consisted of sea water, serum, embryo extract, salts and peptones. They observed limited monolayer growth.
1913	Carrel introduced strict aseptic techniques so that cells could be cultured for long periods
1916	Rous and Jones introduced proteolytic enzyme trypsin for the subculture of adherent cells.
1923	Carrel and Baker developed 'Carrel' or T-flask as the first specifically designed cell culture vessel. They employed microscopic evaluation of cells in culture.
1927	Carrel and Rivera produced the first viral vaccine - Vaccinia.
1933	Gey developed the roller tube technique
1940	The use of the antibiotics penicillin and streptomycin in culture medium decreased the problem of contamination in cell culture
1948	Earle isolated mouse L fibroblasts which formed clones from single cells. Fischer developed a chemically defined medium, CMRL 1066.
1952	Gey established a continuous cell line from a human cervical carcinoma known as HeLa (Helen Lane) cells. Dulbecco developed plaque assay for animal viruses using confluent monolayers of cultured cells.
1954	Abercrombie observed contact inhibition: motility of diploid cells in monolayer culture ceases when contact is made with adjacent cells.
1955	Eagle studied the nutrient requirements of selected cells in culture and established the first widely used chemically defined medium.
1961	Hayflick and Moorhead isolated human fibroblasts (WI-38) and showed that they have a finite lifespan in culture.
1964	Littlefield introduced the HAT medium for cell selection.
1965	Ham introduced the first serum-free medium which was able to support the growth of some cells.
1965	Harris and Watkins were able to fuse human and mouse cells by the use of a virus.
1975	Kohler and Milstein produced the first hybridoma capable of secreting a monoclonal antibody
1978	Sato established the basis for the development of serum-free media from cocktails of hormones and growth factors.
1982	Human insulin became the first recombinant protein to be licensed as a therapeutic agent.
1985	Human growth hormone produced from recombinant bacteria was accepted for therapeutic use.
1986	Lymphoblastoid IFN licensed.
1987	Tissue-type plasminogen activator (tPA) from recombinant animal cells became commercially available.
1989	Recombinant erythropoietin in trial
1990	Recombinant products in clinical trial (HBsAG, factor VIII, HIVgp120, CD4, GM-CSF, EGF, mAbs, IL-2).
1998	Production of cartilage by tissue engineered cell culture by Aigner et al.
2000	Mapping of the human genome.
2007	Use of viral vectors to reprogram adult cells to embryonic state (induced pluripotent stem cells) by Yu et al.
2008	And beyond- Era of induced pluripotent stem cells

(3) Perfusion: by the continuous addition of medium to the culture and the withdrawal of an equal volume of spent (cell-free) medium. Perfusion can be open, i.e. the complete removal of medium from the system or closed which is recirculation of the medium; usually, a secondary vessel is used to regenerate the medium by gassing and pH correction (MacMichael, 1989).

### Continuous-flow culture

This system gives true homeostatic conditions with no fluctuations of nutrients, metabolites or cell number. It depends on the medium entering the culture with a

corresponding withdrawal of medium with cells. It is only suitable for suspension culture cells or monolayer cells growing on micro-carriers. The system has chemostat attached to it. A fixed volume of culture in which medium is fed in, at a constant rate mixed with the cells and then leaves at the same rate (Drake et al., 2002).

### GROWTH KINETICS IN CELL CULTURE

The standard growth kinetics of a culture cycle begins with a lag phase, proceeding through the logarithmic or exponential phase to a stationary phase, and finally to the decline and death of cells. The phases of cell growth in

culture are discussed below:

**The lag phase:** This is the period of adaptation of cells to the new environment. New enzymes are synthesized, a slight increase in cell mass and volume occurs, but there is no increase in cell number. If this phase is prolonged, there could be low inoculum volume and poor inoculum condition (that is, high percentage of dead cells).

**The log or exponential phase:** This is the period of balanced growth in which all cell components grow at the same rate. The cells have adjusted to their new environment and multiply rapidly (exponentially). Growth rate is independent of nutrient concentration, as nutrients are in excess. The composition of the biomass remains constant and the phase results in a straight line graph. Growth (increase in cell numbers or mass) can be defined in the following terms:

**Specific growth rate ( $\mu$ ) or proliferation rate ( $r$ ):** The exponential growth rate is the first order of reaction. The rate of biomass is correlated with the specific growth rate ( $\mu$ ) and the biomass concentration or cell number,  $X$ . A measure of the rapidity of growth has dimension  $T^{-1}$ .

$$dx/dt = \mu \cdot X \quad (1)$$

Where,  $d_x$  = increase in cell mass/number;  $dt$  = time interval, and  $x$  = cell mass/number.

**Doubling time ( $td$ )** that is the time for a population to double in number or mass:

$$td = \frac{\ln 2}{\mu} = \frac{0.693}{\mu} \quad (2)$$

Degree of multiplication ( $n$ ) or number of doublings (that is, the number of times the inoculum has replicated) is defined as:

$$n = 3.34 \log(x/x_0) \quad (3)$$

$$n = 3.34 \times (\log X_0 - \log X) \quad (4)$$

The proliferation rate, ( $r$ ) or specific growth rate ( $\mu$ ) =  $3.32 \times (\log N_F - \log N_i) / T_2 - T_1$  (Griffiths, 1972). Where,  $r$  = Proliferation rate,  $N_F$  = number of Final cell count,  $N_i$  = Number of Initial cell count,  $T_2$  = Time at harvest,  $T_1$  = Initial time.

**Population doubling time (PD):** This is the number of times the inoculums has replicated within 24 hours. It is defined as: (PD) =  $24/r$ , where  $r$  is proliferation rate.

**The deceleration phase:** The exponential phase is followed by deceleration phase, which is the period of unbalanced growth. The growth decelerates due to either

depletion of one or more essential nutrients. There is an accumulation of toxic by-products of growth period of unbalanced nutrients in the medium. Cells undergo internal restructuring to increase their chances of survival.

**The stationary phase:** This phase starts when the net growth rate is zero. Cells may have an active metabolism to produce secondary metabolites. Secondary metabolites are non-growth-related which may be antibiotics like pigments. The decline and death phase is characterized by the living cell population decreasing with time, due to lack of nutrients and toxic metabolic by-products.

The rate of death is defined as =

$$\frac{dN}{dt} = -k_d \cdot N \quad (5)$$

$K_d$  is the cell death constant.  $N$  = Cell number concentration (cell number /L). Figure 1 shows the typical growth curve of a cell population.

## MEDIA REQUIREMENTS FOR CELL CULTURE

### Criteria for selecting culture media

The choice of medium to be used for culture is dependent on the cell type specifics which significantly affects the success of cell culture experiments. The selection of the media also depends on the purpose of the culture and resources available in the laboratory. Different cell types have highly specific growth requirements; therefore, the most suitable media for each cell type must be determined experimentally. In general, it's always good to start with MEM for adherent cells and RPMI-1640 for suspension. Media are the sources of nutrients for the cells in culture. They are rich in essential nutrients such as amino acids, glucose, ions, fructose, and hormones with or without serum. The varieties of artificial culture media available can be grouped into:

### Serum containing media

Serum is a complex mixture of many small and large bio-molecules with different, physiologically balanced growth-promoting and growth-inhibiting activities. Serum performs major functions which are hormonal factors; stimulating cell growth and functions; attachment and spreading factors; transport proteins carrying hormones, minerals, lipids etc. Among other, the serum contains proteins which serve as carriers and protective agents for other molecules. Albumin is a protein in serum which performs a lot of functions. Albumin binds vitamins such as pyridoxal, fatty acids: such as oleic, linoleic, linolenic

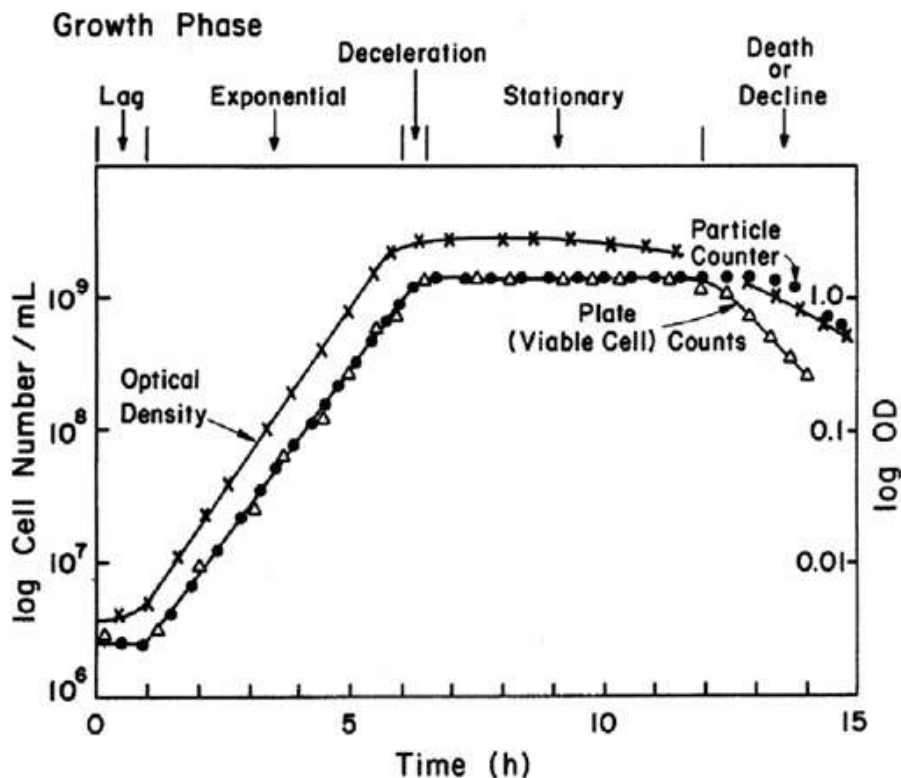


Figure 1. A typical growth curve for a cell population (Griffith, 1972).

arachidonic, myristic and palmitic acids, and ions such as copper. However, the use of serum is limited because of its complexity, expensive and a potential source of adventitious biological contaminants. Eliminating the use of serum in cell culture, it requires an understanding of what it does in *in vitro* systems. Examples of types of serum available for culture are Fetal Bovine Serum, New Calf Serum, Donor Horse Serum, Porcine Serum etc. (Theodore et al., 2005).

### Serum-free media

The development of effective serum-free and serum-replacement medium formulations has become essential for the future growth of the biotechnology. The elimination of animal serum-borne components aims to advance standardization, consistency and to reduce risk of contamination by serum-borne adventitious agents in cell culture processes. Biological industries have developed serum-free formulations for all commercially significant cell and tissue cultures (Brunner et al., 2010). Examples are CHO Cell Culture Media, PER.C6 and 293 Media, Insect Cell Media, Immunology Media, Stem Cell Media, Hybridoma Media, Primary Cell Media. The two categories of serum free media are:

(i) Chemically defined media: A chemically defined medium is one in which the exact chemical composition

is known e.g. Expression media.

(ii) Protein free media: The common examples are hybridoma serum free media, PFHM II is protein-free media.

Table 2 summarizes the list of commercial culture media that are commonly used (Bertheussen, 1993).

### NUTRIENT UTILIZATION IN CELL CULTURE

Nutrient that is likely to be exhausted first is glutamine, and is enzymatically converted (by serum and cellular enzymes) to glutamic acid, leucine and isoleucine. L-Glutamine supports the growth of cells that have high energy demands and synthesize large amounts of proteins and nucleic acids (Le-Bacquer et al., 2001; Cardin et al., 2000). L-Glutamine is an alternative energy source for rapidly dividing cells and cells that use glucose inefficiently. Glucose and pyruvate are other nutrients needed by the cells in culture. When added to a culture at high concentrations, they are stimulatory to maturation of cells and modulation of metabolism of the substrate (Downs et al., 1997). Cysteine is also efficiently utilized by human diploid cells (Banjac et al., 2008). It must be noted that nutrients become growth-limiting before they become exhausted. As the concentration of amino acid falls, the cells find it increasingly difficult to maintain sufficient intracellular pool levels (Duboc and Von

**Table 2.** Commercial culture media and their compositions.

Culture medium	Composition
DMEM	High Glucose - 4 mM L-glutamine 4500 mg glucose/L 1500 mg/L sodium bicarbonate.
RPMI	Modified to contain 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate
McCoy	High Glucose, L-glutamine, Bacto-peptone, Phenol Red, HEPES with or without Sodium Pyruvate.
M 2	Magnesium Sulfate , Potassium Chloride, Potassium Phosphate, Sodium Bicarbonate, Sodium Chloride, Albumin, Bovine Fraction, D-Glucose, HEPES, Phenol, Pyruvic Acid, DL-Lactic Acid.
Nutrient Mixture F-10 (Ham's F-10)	Sodium bicarbonate, without L- glutamine; supplement with 0.146 gm/L L-glutamine.
Nutrient Mixture F-12(Ham's F-12)	L-glutamine and Sodium bicarbonate
Minimum Essential Medium Eagle	Earle's salts, L-glutamine and Sodium bicarbonate, Sterile-filtered, Endotoxin tested.

**Table 3.** List of main equipment for cell culture.

Type of Equipment	Function
Bio Safety Cabinets	It offers protection from contaminants during culture.
CO <sub>2</sub> Incubators	Cells are grown in an atmosphere of 5%-10% CO <sub>2</sub> . It keeps constant level of humidity
Microscopes	Inverted microscopes are used for this purpose.
Culture vessels	These consist of petri dishes, multi-well plates, microtitre plates, roller bottles, screw cap flasks T-25, T-75, T-150.
Centrifuges	Cells are centrifuged at low temperature and low speed.
Freezer	For freezing and short term storage
Hemocytometer	To determine the cell counts before or after culture.
Water bath with shaker	For cell dissociation and trypsinization
Liquid N <sub>2</sub> Cylinder	For long-term cryopreservation
pH meter	To determine the pH of the medium.

Stockar, 2000). This is exaggerated in monolayer cultures because as the cells become more tightly packed together, the surface area which is available for nutrient uptake becomes smaller. Glucose is often another limiting factor as it is destructively utilized by cell rather than adding high concentrations at the beginning, it is more beneficial to supplement after 2-3 days. In order to maintain a culture some additional feeding often has to be carried out either by complete, partial media changes or by perfusion. The efficiency of medium changes is probably due to the high extracellular concentration of nutrients it provides, thus stimulating a further replicative cycle. Many cell types are either totally dependent upon or can only perform optimally when certain growth factors are present. Cell aggregation is often a problem in suspension cultures. Media lacking calcium and magnesium ions have been designed specifically for suspension cells because of the role of these ions in attachment. This problem has also been overcome by including very low levels of trypsin in medium (Makkar et al., 2011; Lugo et al., 2008; Baumann and Doyle, 1979). The main equipment required for cell culture is listed in Table 3.

#### **Other conditions for cell culture: effect of pH, temperature and oxygen on cell growth**

In addition to nutrients, the pH of the growth medium is also important for cell growth rate and cell density. The optimal growth pH for most cells is near neutral. Cells can grow reasonably well over a range of pH 5.5 to 8.5. Extreme pH beyond this range will significantly decrease the cell growth rate and may sometimes even cause cell death. pH is another limiting factor for cell growth in addition to nutrition exhaustion and accumulation of toxic metabolites. The medium's pH is determined by medium compositions, buffers, cellular metabolites, and aeration conditions. Cells produce large quantities of acetic acid if the growth medium contains little or no oxygen causing the growth medium to reach pH 4 or lower. Acetic acid is the major metabolic inhibitor under anaerobic growth condition. With proper aeration, cells will be able to use many organic acids as carbon sources and the pH of the growth medium will be maintained at near neutral or basic ranges. Cells cannot grow well at temperatures higher than 42°C. They can tolerate lower temperatures with lower growth rate. Temperature range from 15 to

30°C is the most optimal range for most cells depending on the cell type and species of animals. Cell growth stops when the medium is kept at 4°C or shifted above 37°C (Yang and Xiong, 2012).

### **CRYOPRESERVATION AND STORAGE**

Liquid Nitrogen is often used to preserve tissue culture cells, either in the liquid phase (-196°C) or in the vapour phase (-156°C). Freezing can be lethal to cells due to the effects of damage by ice crystals, alterations in the concentration of electrolytes, dehydration, and changes in pH. To minimize the effects of freezing, several precautions are taken. First, a cryoprotective agent which lowers the freezing point, such as glycerol or dimethylsulfoxide (DMSO) is added. A typical freezing medium is 90% serum and 10% DMSO (Oyeleye and Omitogun, 2007).

In addition, it is best to use healthy cells that are growing in log phase and to replace the medium 24 h before freezing. Also, the cells are slowly cooled from room temperature to -80°C to allow the water to move out of the cells before it freezes. The optimal rate of cooling is 1 to 3°C per minute. Some laboratories use isopropanol at room temperature and the freezing vials containing the cells are placed in the container and the container is placed in the -80°C freezer. The effect of the isopropanol is to allow the tubes to come to the temperature of the freezer slowly, at about 1°C per minute. To maximize recovery of the cells when thawing, the cells are warmed very quickly by placing the tube directly from the liquid nitrogen container into a 37°C water bath with moderate shaking. As soon as the last ice crystal is melted, the cells are immediately diluted into pre-warmed medium. Cultures should be examined daily, by observing the morphology, the colour of the medium and the density of the cells. A tissue culture logbook should be maintained and it should contain: the name of the cell line, the medium components and any alterations to the standard medium, the dates on which the cells were split and/or fed, a calculation of the doubling time of the culture and any observations relative to the morphology (Jacob and Allison, 2009).

### **CELL LINE IDENTIFICATION**

Cell line identification is done to determine if there is presence or absence of cross-contamination. It also confirms the origin of species of the cell line. It detects the transformed cells and evaluates if there are genetic instabilities. The following techniques discussed below are used to prove the integrity of the cultured cells.

#### **Morphology and STR analyses**

Observation of morphology is the most direct technique

to characterize cell lines. The study of size, shape and structure of cells can authenticate the type and origin of a particular cell line. Most cells can be divided into five basic categories based on their morphology. The structures are fibroblastic, epithelial-like, lymphoblast-like, endothelial and Neuronal (Figures 2, 3, 4, 5 and 6). Short tandem repeats (STR) profile of a reference sample and other known cell line STR profile can be compared. STR is repetitive sequence elements 3 to 7 base pairs of DNA long scattered throughout the human genome. By amplifying and analyzing these polymorphic loci, comparing the resulting STR profile to that of a reference sample, the origin of biological samples such as cells or tissues can be identified and verified (Gill, 2002; Chatterjee, 2007).

#### **Chromosomal and Karyotyping analyses:**

This is done to detect the presence of genetic abnormalities within the cell. A karyotype is the number and appearance of chromosomes in the nucleus of eukaryotic cells. The chromosomes are depicted in a standard format known as Karyogram. The karyotyping is done to determine species identification (ACOG, 2007).

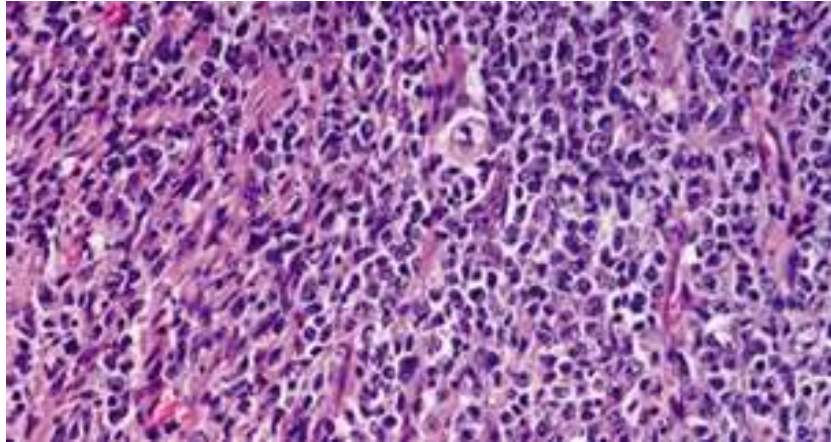
#### **Isoenzyme analysis**

Isoenzyme analysis is based on the existence of enzymes with similar or identical specificity, but different molecular structure. It is used to study the pattern of migration of isoenzymes present in cell lysates following electrophoresis using agarose gels. Examples of isoenzymes available are; Aspartate aminotransferase, Glucose-6-phosphate dehydrogenase, Lactate dehydrogenase, Malate dehydrogenase (Steube et al., 1995).

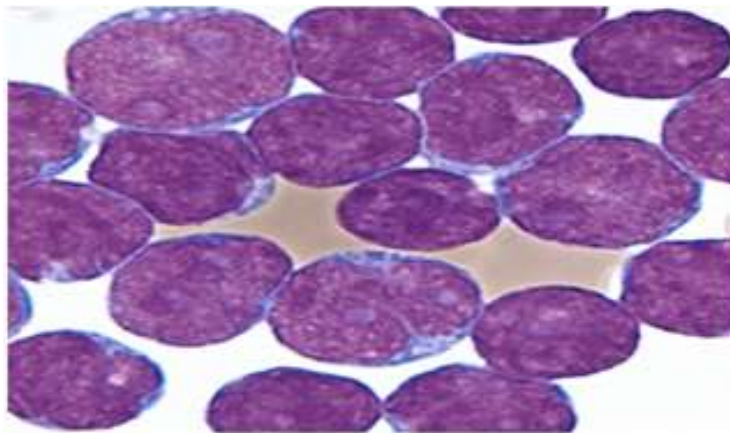
#### **ELISA**

The enzyme-linked immuno-sorbent assay (ELISA) is a common laboratory technique which is used to measure the concentration of a substance (usually antibodies or antigens) in solution. The basic ELISA, or enzyme immunoassay (EIA), is distinguished from other antibody-based assays because of its separation of specific and non-specific interactions which occur via serial binding to a solid surface. Usually a polystyrene multi-well plate can be achieved due to the quantitative results that can be obtained (Braitbard et al., 2006). The steps of the ELISA result in a colored end product correlates to the amount of a substance present in the original sample. ELISAs were first developed in the early 1970s as a replacement for radio-immunoassays. They remain in wide use in their original format and in expanded format with modifications that allow multiple analyses per well, highly sensitive readouts, and direct cell-based output (Braitbard et al., 2006).

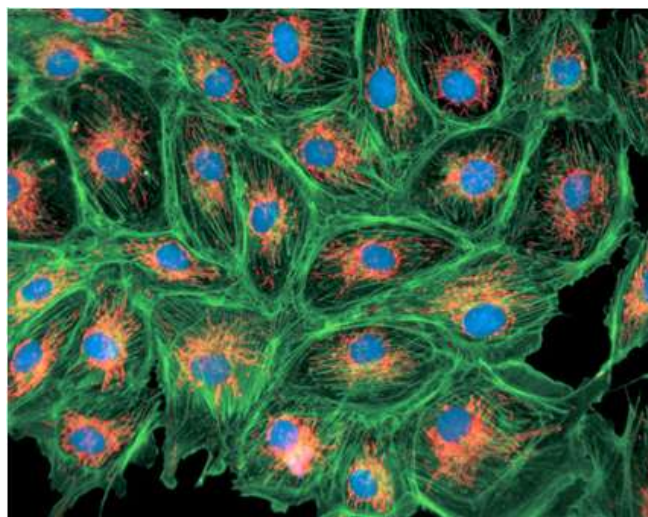




**Figure 2.** Epithelial cells are polygonal in shape with more regular dimensions, and attached to a substrate in discrete patches.

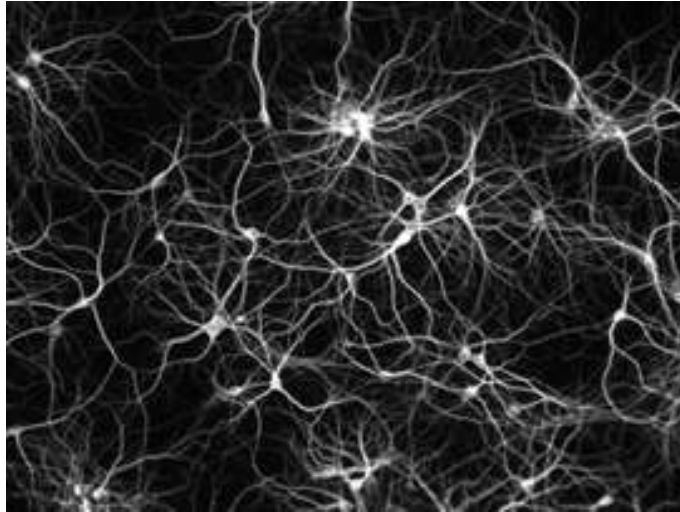


**Figure 3.** Lymphoblastic cells are spherical in shape and usually grown in suspension without attaching to a surface.



**Figure 4.** Endothelial cells are flat in shape having central nucleim of about 1-2  $\mu\text{m}$  thick and some 10-20 $\mu\text{m}$  in diameter.





**Figure 5.** Neuronal cell lines can be with or without axons.



**Figure 6.** Fibroblastic cells are bipolar or multipolar, have elongated shapes and grown attached to a surface.

### Biochemical tests

A large number of cell lines and strains can be shown to derive from a particular tissue or tumour by the presence of specific synthetic abilities or metabolic pathways. The human trophoblastic are cell lines isolated from a malignant gestational choriocarcinoma of a foetal placenta. Interestingly, the line has been shown to secrete a spectrum of placental hormones, including human chorionic gonadotrophin, placental lactogenic, oestrogen, oestradiol, oestriol and progesterone in culture (Soule et al., 1973). Biochemical tests are the tests used for the identification of cells based on the differences in the biochemical activities of different cell

functions. These differences in carbohydrate metabolism, protein metabolism, fat metabolism, production of certain enzymes, hormones and ability to utilize a particular compound, etc. help to identify them by their biochemical tests (Oyeleye et al., 2016).

### Tests for microbial contamination

These tests are suitable for detection of most microorganisms that would be expected to survive as contaminants in cell lines or culture fluids. The common examples of contaminants that could affect culture are bacteria, fungi, mycoplasma and viruses. The following

tests should be carried out to avoid any microbial contaminant: microbial environmental aseptic monitoring, container integrity testing, pre-sterilization bio-burden testing, media filtering before use and sterility testing (Sirna et al., 2010).

### Tests for intra-species cross-contamination

A quiet number of cell lines are being developed; there is a high risk of intra-species cross-contaminants in the laboratory. The problem is common, especially in laboratories where many different cell lines of human and murine origins are being developed. Tests for polymorphic isoenzymes, surface marker antigens and unique karyology are all important tools to detect cellular cross-contamination within a given species (Chatterjee, 2007). The other methods for identification of cell lines include Giemsa binding, tissue-specific antigen, cell type specific marker etc.

### CONCLUSION

Animal cell culture is important to all fields of bioscience; especially from medicine to agriculture. It is an important tool to study cell specific functions, physiology and biochemical components. The major advantage of cell culture is its consistency and reproducibility of results that can be obtained from using clonal cells. They serve as building blocks and stem cells for bioscience research and biological repairs. Priority should be given to developing good cell culture laboratories especially in developing countries where they are not yet fully established.

### Conflict of Interests

The authors have not declared any conflict of interests.

### ACKNOWLEDGEMENTS

The authors are grateful to the technical staff of the Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife for their cooperation and efforts to provide information used in this article and Wesley University Ondo, Ondo State for their financial assistance.

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