CELL CULTURE

HISTORY

- Ross Harrison (1907)- frog embryo nerve fiber outgrowth in vitro.
- Lewis (1911)- made the first liquid media consisted of sea water, serum, embryo extract, salts and peptones.
- Rous & Jones (1916)- introduced proteolytic enzyme trypsin for the subculture of adherent cells.
- Keilova (1948)- use of antibiotics in tissue culture.
- Gey et al. (1952)- First Human cell line HeLa established.
- Eagle (1955)- established the first widely used chemically defined medium (DMEM).
**HISTORY**

- Kohler & Milstein (1975)- produced the first hybridoma capable of secreting a **monoclonal antibody**.
- Rheinwald & Green (1975)- Skin culture.
- Ham & McKeehan (1978) - Serum free media.
- Butler (1991)- Industrial scale culture of transfected cells for biopharmaceuticals.
- Freshney (2004)- Exploitation of tissue engineering.

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**WHAT FOR CELL CULTURE?**

- **Research**
  - To overcome problems in studying cellular behavior such as:
    - confounding effects of the surrounding tissues
    - variations that might arise in animals under experimental stress
  - Reduce animal use

- **Commercial or large-scale production**
  - Production of cell material: vaccine, antibody, hormone
Types of Tissue Culture

1. Cell culture: a culture derived from dispersed cells

2. Explant culture: culture of tissue fragments

3. Organ culture: a three-dimensional culture of undisaggregated tissue retaining some of the histological features of the tissue

4. Histotypic culture: cells have been grown to re-create a 3-D structure with tissue-like cell density

5. Organotypic culture: recombining cells of different lineages
Types of tissue culture

Organotypic culture

Histotypic culture

“two cell type”

“one cell type”

Table 1-4 Properties of Different Types of Culture

<table>
<thead>
<tr>
<th>Category</th>
<th>Organ Culture</th>
<th>Explante culture</th>
<th>Cell Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Embryonic organs, adult tissue fragments</td>
<td>Tissue fragments</td>
<td>Disaggregated tissue, primary culture, propagated cell line</td>
</tr>
<tr>
<td>Effort</td>
<td>High</td>
<td>Moderate</td>
<td>Low</td>
</tr>
<tr>
<td>Characterization</td>
<td>Easy, histology</td>
<td>Cytology and makers</td>
<td>Biochemical, molecular, immunological, and cytochemical assays</td>
</tr>
<tr>
<td>Histology</td>
<td>Informative</td>
<td>Difficult</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Biochemical differentiation</td>
<td>Possible</td>
<td>Heterogeneous</td>
<td>Low</td>
</tr>
<tr>
<td>Propagation</td>
<td>Not possible</td>
<td>Possible</td>
<td>Standard procedure</td>
</tr>
<tr>
<td>Replicate sampling, reproducibility</td>
<td>High intersample variation</td>
<td>High intersample variation</td>
<td>Low level of variation</td>
</tr>
<tr>
<td>Homogeneity</td>
<td>Difficult</td>
<td>Difficult</td>
<td>Easy, many techniques available</td>
</tr>
<tr>
<td>Quantitation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ISOLATION OF TISSUES

Must comply with local legislation and medical ethical rules.
Sterilize the site with 70% alcohol.
Remove tissue aseptically.
Transfer to the laboratory in transport medium
If delay in transporting to lab, keep at 4°C for up to 72 hours.

PRIMARY CULTURE

- cells that are placed in culture directly from the tissue of origin.

- Isolation of tissues-Mechanical & Enzymatic:
  - Mechanical methods- sieving, syringing, vigorous pipetting
  - Enzymatic methods- warm trypsin, cold trypsin & collagenase treatment
TYPES OF PRIMARY CELL CULTURE

Mouse embryos
Chick embryos
Human biopsy materials
Transplantable animal tumour
Chick embryo organ (brain, heart, lungs, liver, kidney, spinal cord, skin, muscle)
Mouse, mammals, Embryo Eggs
(best for TC: embryo, young because stage of differentiation)

Finely cut tissue or explant

Primary explant

organ

Finely cut

Cell culture

• Mouse, mammals, Embryo Embryonated Eggs
(best: for TC: embryo, young) because stage of differentiation)

Finely cut tissue or explant

Enzymic digestion

Grow in media
-Explants
-Explants with outgrowth

Grow in media
- monolayer
- suspension cells

organ
**PRIMARY VS CELL LINE**

- Primary culture – freshly isolated from tissue source

- Cell line – culture that has been passaged
  - Finite cell line: dies after several sub-cultures
  - Continuous cell line: transformed ‘immortal’ from
    - tumor cells.
    - viral oncogenes
    - chemical treatments.

  the disadvantage of having retained very little of the original *in vivo* characteristics

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**PASSAGING OR SUB-CULTURE**

Cell dissociated from flask

Split 1 in 2
**Transformation VS Transfection**

Transformation
Spontaneous or induced permanent phenotypic changes resulting from change in DNA and gene expression

Transfection
Introduction of DNA into a cell (like viral DNA)

---

**Tissue Culture Application (I)**

1. **INTRACELLULAR ACTIVITY**
   - DNA transcription, protein synthesis, energy metabolism, drug metabolism, cell cycle, differentiation, apoptosis

2. **INTRACELLULAR FLUX**
   - RNA processing, hormone receptors, metabolite flux, calcium mobilization, signal transduction, membrane trafficking

3. **PHARMACOLOGY**
   - Drug action, ligand receptor interactions, drug metabolism, drug resistance

4. **CELL-CELL INTERACTION**
   - Morphogenesis, pericellular control, cell proliferation kinetics, metabolic cooperation, cell adhesion and motility, matrix interaction, invasion

5. **TOXICOLOGY**
   - Inflammation, cytotoxicity, mutagenesis, carcinogenesis, irritation, inflammation

6. **TISSUE ENGINEERING**
   - Tissue constructs, matrices and scaffolds, stem cell sources, propagation, differentiation

7. **GENOMICS**
   - Genetic analysis, transfection, infection, transformation, immortalization, senescence

8. **IMMUNOLOGY**
   - Cell surface epitopes, hybridomas, cytokines and signaling, inflammation

9. **CELL PRODUCTS**
   - Proteomics, secretion, biotechnology, biomarker design, product harvesting, downstream processing
**Tissue Culture Application (II)**

1. Production of antiviral vaccines
2. Understanding of neoplasia (cancer research)
3. Transfer of DNA to the cultured cells (or siRNA)
4. Monoclonal antibody production (immunology)
5. Production of human growth hormone, insulin, interferon
6. Stem cell culture differentiate into neurons
7. Implanting normal fetal neurons into patients with Parkinson diseases
8. Homografting and reconstructive surgery using individual’s own cells (tissue engineering)

**Advantage of tissue culture**

1. Control of the environment: Control the physiochemical environment very precisely. However, cell line still require supplementation of the medium with serum or other poorly defined constituents, e.g: serum, supplementation, matrix…
ADVANTAGE OF TISSUE CULTURE

2. Cell line homogeneity:
   After one or two passages, cultured cell lines assume a homogeneous constitution.
   
   the cells are randomly mixed at each transfer and the selective pressure of the culture condition tends to produce a homogeneous culture of the most vigorous cell type.

ADVANTAGE OF TISSUE CULTURE

3. Reagent saving: “Less reagent is required than for injection in vivo, where 90% is lost by excretion and distribution to tissues other than the interested cells under study”

4. Reduction of animal use: In vitro modeling of in vivo conditions
### Table 1-2: Advantages of Tissue Culture

<table>
<thead>
<tr>
<th>Category</th>
<th>Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Physico-chemical environment</td>
<td>Control of pH, temperature, osmolarity, dissolved gases (O2 &amp; CO2)</td>
</tr>
<tr>
<td>2) Physiological conditions</td>
<td>Control of hormone and nutrient concentrations</td>
</tr>
<tr>
<td>Microenvironment</td>
<td>Regulation of matrix, cell–cell interaction, gaseous diffusion</td>
</tr>
<tr>
<td>Cell line homogeneity</td>
<td>Availability of selective media, cloning</td>
</tr>
<tr>
<td>Characterization</td>
<td>Cytology and immunostaining are easily performed</td>
</tr>
<tr>
<td>Preservation</td>
<td>Can be stored in liquid nitrogen</td>
</tr>
<tr>
<td>Validation &amp; accreditation</td>
<td>Origin, history, purity can be recorded</td>
</tr>
<tr>
<td>Replicates and variability</td>
<td>Quantitation is easy</td>
</tr>
<tr>
<td>Reagent saving</td>
<td>Reduced volumes, direct access, lower cost</td>
</tr>
<tr>
<td>Control of C × T</td>
<td>Ability to define dose, concentration, and time</td>
</tr>
<tr>
<td>Mechanization</td>
<td>Available with microtitation and robotics</td>
</tr>
<tr>
<td>Reduction of animal use</td>
<td>Cytotoxicity and screening of pharmaceuticals, cosmetics, etc.</td>
</tr>
</tbody>
</table>

### Table 1-3: Limitations of Tissue Culture

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necessary expertise</td>
<td>Handling, Chemical contamination, Microbial contamination, Cross contamination</td>
</tr>
<tr>
<td>Environmental control</td>
<td>Workplace, Incubation, pH control, Containment and disposal of biohazards</td>
</tr>
<tr>
<td>Quantity and cost</td>
<td>Capital equipment, Consumables, Medium, serum, plastics, Heterogeneity, variability</td>
</tr>
<tr>
<td>Genetic instability</td>
<td>Deciliation, Adaptation, Selection, Expression of markers</td>
</tr>
<tr>
<td>Phenotypic instability</td>
<td>Histology, cytology, Geometry and microenvironment</td>
</tr>
<tr>
<td>Identification of cell type</td>
<td></td>
</tr>
</tbody>
</table>
Sources of Contamination

- Bacteria
- Fungi
- Mold
- Yeast
- Mycoplasma
- Other cell types
- Free organisms, dust particles or aerosols
- Surfaces or equipment

CROSS CONTAMINATION

"Many cell lines in common use are not what they are claimed to be, but have been cross-contaminated with HeLa or other growing cell line"
Cross-Contaminated cell lines

"HeLa cell": The line was derived from cervical cancer cells taken on February 8, 1951. By 1954 Jonas Salk developed a vaccine for polio using these cells.

George Otto Gey

Henrietta Lacks

海拉細胞

是被人類乳突病毒第18型（Human papillomavirus 18）轉化的。海拉細胞生長奇快，甚至超越一般癌細胞。

經歷細胞分裂時可維持端粒酶活性以維持端粒長度。

已證實海拉細胞系難以控制。此細胞系有時會污染同一實驗室的其他細胞培養物（cell culture），干擾生物學的研究。

被George Gey分送給眾研究單位，並用作癌症模式細胞（model cancer cells）研究。HeLa細胞系也被用作研究細胞信號傳導（cellular signal transduction）。
## Cross-Contamination

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Species</th>
<th>Cell Line</th>
<th>Cross-Contaminant</th>
<th>Specimen</th>
<th>Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>207</td>
<td>Human</td>
<td>Renal fibroblasts</td>
<td>MRC-5</td>
<td>Human</td>
<td>Renal carcinoma</td>
</tr>
<tr>
<td>293T</td>
<td>Human</td>
<td>Cervical carcinoma</td>
<td>HeLa</td>
<td>Human</td>
<td>Cervical carcinoma</td>
</tr>
<tr>
<td>293T-780</td>
<td>Human</td>
<td>Cervical carcinoma</td>
<td>HeLa</td>
<td>Human</td>
<td>Cervical carcinoma</td>
</tr>
<tr>
<td>313T</td>
<td>Human</td>
<td>Cervical carcinoma</td>
<td>HeLa</td>
<td>Human</td>
<td>Cervical carcinoma</td>
</tr>
<tr>
<td>A549</td>
<td>Human</td>
<td>Lung carcinoma</td>
<td>NCI-H1299</td>
<td>Human</td>
<td>Lung carcinoma</td>
</tr>
<tr>
<td>BEAS</td>
<td>Human</td>
<td>Ameloblastoma</td>
<td>HeLa</td>
<td>Human</td>
<td>Cervical carcinoma</td>
</tr>
<tr>
<td>BEAS-2B1</td>
<td>Human</td>
<td>Ameloblastoma</td>
<td>HeLa</td>
<td>Human</td>
<td>Cervical carcinoma</td>
</tr>
<tr>
<td>BEAS-111</td>
<td>Human</td>
<td>Ameloblastoma</td>
<td>HeLa</td>
<td>Human</td>
<td>Cervical carcinoma</td>
</tr>
<tr>
<td>BEAS-361</td>
<td>Human</td>
<td>Ameloblastoma</td>
<td>HeLa</td>
<td>Human</td>
<td>Cervical carcinoma</td>
</tr>
<tr>
<td>BEAS-69.1</td>
<td>Human</td>
<td>Osteosarcoma</td>
<td>SNU-601</td>
<td>Human</td>
<td>Bladder carcinoma</td>
</tr>
<tr>
<td>BEAS-180</td>
<td>Human</td>
<td>Osteosarcoma</td>
<td>SNU-601</td>
<td>Human</td>
<td>Bladder carcinoma</td>
</tr>
<tr>
<td>BEAS-34-2</td>
<td>Human</td>
<td>Osteosarcoma</td>
<td>SNU-601</td>
<td>Human</td>
<td>Bladder carcinoma</td>
</tr>
<tr>
<td>BEAS-64-1</td>
<td>Human</td>
<td>Osteosarcoma</td>
<td>SNU-601</td>
<td>Human</td>
<td>Bladder carcinoma</td>
</tr>
<tr>
<td>BEAS-64-2</td>
<td>Human</td>
<td>Osteosarcoma</td>
<td>SNU-601</td>
<td>Human</td>
<td>Bladder carcinoma</td>
</tr>
<tr>
<td>BEAS-64-3</td>
<td>Human</td>
<td>Osteosarcoma</td>
<td>SNU-601</td>
<td>Human</td>
<td>Bladder carcinoma</td>
</tr>
<tr>
<td>BEAS-64-4</td>
<td>Human</td>
<td>Osteosarcoma</td>
<td>SNU-601</td>
<td>Human</td>
<td>Bladder carcinoma</td>
</tr>
<tr>
<td>BEAS-64-5</td>
<td>Human</td>
<td>Osteosarcoma</td>
<td>SNU-601</td>
<td>Human</td>
<td>Bladder carcinoma</td>
</tr>
</tbody>
</table>

### THE CELL CULTURE ENVIRONMENT

- [Image of a cell culture environment]
FACTORS AFFECTING CELL BEHAVIOUR IN VIVO

- The local micro-environment
- Cell-cell interactions
- Tissue architecture
- Tissue matrix
- Tissue metabolites
- Locally released growth factor and hormones

BIOLOGY OF CULTURE CELLS

Cell growth and differentiation in the culture depends on:
- The nature of cells
- The culture environment:
  - the nature of the substrate on which cell grow
  - the physicochemical and physiological constitution of culture medium
  - the constitution of gas phase
  - the incubation temperature
  - the cell-cell and cell-matrix interaction
**Physical Environment for Culture**

- To provide an environment that mimics the in vivo environment of that specific cell type.

- Provides an appropriate temperature, pH, oxygen, and CO\(_2\) supply, surface for cell attachment, nutrient and vitamin supply.

- Protection from toxic agents, the hormones and growth factors that control the cell's state of growth and differentiation.

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**Culture Surface**

- Most adherent cells require attachment to proliferate
- Change charge of the surface
  - Poly-L-lysine
- Coating with matrix proteins
  - Collagen, laminin, gelatin, fibronectin
**Medium**

A growth medium or culture medium is a liquid or gel designed to support the growth of cells.

**Natural and artificial.**

<table>
<thead>
<tr>
<th>TYPES OF CELL CULTURE MEDIA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Media Type</strong></td>
</tr>
<tr>
<td>Natural media</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Artificial media</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
**Natural Media**

Very useful.

Lack of knowledge of the exact composition of these natural media.

- Clots - Plasma clots (male fowl)
- Biological Fluids - Amniotic, Ascitic fluid, serum
- Tissue extracts - Chicken and bovine embryo extract

**Artificial Media**

- Defined & Complex media
  - Immediate Survival
  - Prolonged Survival
  - Indefinite growth
  - Specialized function

- Classes
  - Serum containing media
  - Serum free media
  - Chemically defined Media
  - Protein free media
BASIC COMPONENTS OF CULTURE MEDIA

Culture media (as a powder or as a liquid) contains:
- amino acids
- Glucose
- Salts
- Vitamins
- Other nutrients

The requirements for these components vary among cell lines, and these differences are partly responsible for the extensive number of medium formulations.

COMMON CELL CULTURE MEDIA

- Eagle’s Minimum Essential Medium (EMEM)
- Dulbecco’s Modified Eagle’s Medium (DMEM)
  - Low glucose
  - High glucose
- RPMI-1640
- Ham’s Nutrient Mixtures
- DMEM/F12
- Iscove’s Modified Dulbecco’s Medium (IMDM)
EAGLE'S MINIMAL ESSENTIAL MEDIUM (EMEM)

Developed by Harry Eagle. It contains:

- amino acids
- salts (calcium chloride, potassium chloride, magnesium sulfate, sodium chloride, and monosodium phosphate)
- glucose
- vitamins (folic acid, nicotinamide, riboflavin, B\textsubscript{12})

DULBECCO’S MODIFIED EAGLE'S MEDIUM (DMEM)

Contains approximately four times as much of the vitamins and amino acids present in the original formula and two to four times as much glucose.

Additionally, it contains iron and phenol red. DMEM is suitable for most types of cells, including human, monkey, hamster, rat, mouse, chicken and fish cells.
**ROSWELL PARK MEMORIAL INSTITUTE MEDIUM 1640 (RPMI 1640)**

It has traditionally been used for growth of human lymphoid cells.

This medium contains a great deal of phosphate and is formulated for use in a 5% carbon dioxide atmosphere.

RPMI 1640 has traditionally been used for the serum-free expansion of human lymphoid cells.

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**CRITERIA FOR SELECTING MEDIA**

- Immediate survival
- Prolonged survival
- Indefinite growth
- Specialized functions

It’s always good to start with MEM for adherent cells and RPMI-1640 for suspension cells
<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Morphology</th>
<th>Species</th>
<th>Medium</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa B</td>
<td>Epithelial</td>
<td>Human</td>
<td>MEM + 2mM Glutamine + 10% FBS + 1% Non Essential Amino Acids (NEAA)</td>
<td>Tumourigenicity and virus studies</td>
</tr>
<tr>
<td>HL60</td>
<td>Lymphoblast</td>
<td>Human</td>
<td>RPMI 1640 + 2mM Glutamine + 10-20% FBS</td>
<td>Differentiation studies</td>
</tr>
<tr>
<td>3T3 clone A31</td>
<td>Fibroblast</td>
<td>Mouse</td>
<td>DMEM + 2mM Glutamine + 5% New Born Calf Serum (NBCS) + 5% FBS</td>
<td>Tumourigenicity and virus studies</td>
</tr>
<tr>
<td>COS-7</td>
<td>Fibroblast</td>
<td>Monkey</td>
<td>DMEM + 2mM Glutamine + 10% FBS</td>
<td>Gene expression and virus replication studies</td>
</tr>
<tr>
<td>CHO</td>
<td>Epithelial</td>
<td>Hamster</td>
<td>Ham's F12 + 2mM Glutamine + 10% FBS</td>
<td>Nutritional and gene expression studies</td>
</tr>
<tr>
<td>HEK 293</td>
<td>Epithelial</td>
<td>Human</td>
<td>EMEM (EBSS) + 2mM Glutamine + 1% Non Essential Amino Acids (NEAA) + 10% FBS</td>
<td>Transformation studies</td>
</tr>
<tr>
<td>HUVEC</td>
<td>Endothelial</td>
<td>Human</td>
<td>F-12 K + 10% FBS + 100 µg/ml Heparin</td>
<td>Angiogenesis studies</td>
</tr>
<tr>
<td>Jurkat</td>
<td>Lymphoblast</td>
<td>Human</td>
<td>RPMI-1640 + 10% FBS</td>
<td>Signaling studies</td>
</tr>
</tbody>
</table>

**SERUM CONTAINING MEDIA**

- MEM, DMEM, M199, F12, DMEM/F12, etc
- Provide plasma protein, peptides, lipids, carbohydrates, minerals and enzyme
- Hormones (cortisone, insulin and testosterone and prostaglandin)
- Growth factors (PDGF, TGF-p, epidermal growth factors etc)
- Supply protein (fibronectin, spreading factor)
- Binding factors (albumin, transferrin)
- Increase viscosity of medium
- Protease inhibitor
- Buffer
- Minerals (Na, K, Fe, Zn, and Cu etc)
DISADVANTAGES OF USING SERUM

1. Serum may inhibit growth of some cell types, e.g., epidermal keratinocytes.

2. Serum may contain some cytotoxic or potentially cytotoxic constituents.

3. There is a large variation in serum quality from one batch to another.

4. Some growth factors may be inadequate for specific cell types and may need supplementation.

5. It interferes with downstream processing when cell cultures are used for production of biochemicals.

SERUM FREE MEDIA

- Analytical approach
- Synthetic approach
- Limiting factor approach

- Defined Media
  EMEM, DME, Ham's F12, CMRL 1066, RPMI 1640, Iscove's modified Dulbecco's medium (IMDM)
SERUM-FREE MEDIA - ADVANTAGES

1. Improved **reproducibility** of results from different laboratories and over time since variation due to batch change of serum is avoided.

2. **Easier downstream processing** of products from cultured cells.

3. Toxic effects of serum are avoided.

4. Biassays are free from interference due to serum proteins.

5. There is **no danger of degradation** of sensitive protein by serum proteases.

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SERUM-FREE MEDIA - DISADVANTAGES

1. Most serum-free media are specific to one **cell type**.

2. Reliable serum-free preparations, for most of the media formulations are not available commercially.

3. A **greater control of pH**, temperature etc. is necessary as compared to that with serum containing media.

4. **Growth rate and the maximum cell density** attained are lower than those with serum containing media.
**PREPARATION AND STERILIZATION OF MEDIUM**

- The various media constituents and other reagents used in cell cultures must be carefully sterilized either by autoclaving or by filtration.

- Heat stable *constituents like* water, salts, supplements like peptone or tryptose etc. are autoclaved at 121°C for 20 min.

- But heat labile constituents like serum, trypsin, proteins, growth factors etc. must be sterilized by filtration through a 0.2 mm porosity membrane filter.

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**THE GAS PHASE**

- **Oxygen**
  - Aerobic metabolism
  - Atmospheric 20%
  - Tissue levels between 1-7%

- **Carbon dioxide**
  - Buffering
PH CONTROL

- Physiological pH 7
- pH can affect
  - Cell metabolism
  - Growth rate
  - Protein synthesis
  - Availability of nutrients
- CO₂ acts as a buffering agent in combination with sodium bicarbonate in the media

TEMPERATURE AND HUMIDITY

- Normal body temperature 37°C
- Humidity must be maintained at saturating levels as evaporation can lead to changes in
  - Osmolarity
  - Volume of media and additives
EQUIPMENT AND MATERIALS

Cell Culture Equipment

• The specific requirements of a cell culture laboratory depend mainly on the type of research conducted.

• 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.
**Basic equipment**

- Cell culture hood
- Incubator
- Centrifuge
- Refrigerator & freezer
- Liquid nitrogen
- Cell counter & microscope

**Expanded & additional equipment**

- PH meter
- Pipette
- Waste containers
- Syringes and tubes
Laminar- flow Hood

- There are three different types of Biological Safety Cabinets (BSC) which are classified based on

  §§ effectiveness of personnel.
  §§ environment
  §§ product protection
  §§ airflow dynamics.

- Usually equipped with UV light for sterilization of the work surface – use it BEFORE and AFTER not during work.

- Hood is not a storage area!
Laminar-Flow Hood. A peristaltic pump, connected to a receiver vessel, is shown on the right side below the hood, with a foot switch to activate the pump. The suction line from the pump leads to the work area, and a delivery tube from a gas mixer provides a supply of CO$_2$ mixed in air.

• Biological Safety Cabinets (BSC)
  • CLASS I
  • CLASS II
  • CLASS III
BSC CLASS I

- ventilated cabinets which provide protection for the researcher and the environment.

- They are normally characterized by a non-recirculated, inward airflow away from the operator via a limited fixed access opening.

High efficiency partial air (hepa)

- Theoretically it can remove at least 99.97% of dust, pollen, mold, bacteria and any airborne particles with a size of 0.3 micrometres (µm) at 85 litres per minute (Lpm).

 to filter out highly hazardous aerosols such as those that are radioactive, biohazardous and highly toxic (e.g. carcinogens). In the event of a nuclear, biological or chemical outbreak.
Bsc class II

- provide personnel, product and environmental protection.

- classified into types based on the amount of air that is recirculated in the cabinet

---

Bsc class III (Glove box)

- provide the ultimate protection for personnel, product and the environment.

- They are characterized by a completely enclosed, gas tight, negative pressure, HEPA filtered, ventilated workspace which is accessed through rubber gloves which are attached to the unit.
Incubator

- The purpose of the incubator is to provide the appropriate environment for cell growth.

- The incubator should be large enough for your laboratory needs and should have temperature control to within ±0.2°C.

- Stainless steel incubators allow easy cleaning and provide corrosion protection, especially if humid air is required for incubation.

- **TYPE**: Dry and Humid CO2

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FIGURE 4.12 CO$_2$ Incubator. Galaxy 170R fanless CO$_2$ incubator.
(a) Exterior with LCD display panel.
(b) Interior showing shelving and water tray in place; and
(c) showing smooth easily cleaned interior with removable racking that does not penetrate the stainless steel lining.
FIGURE 4.11 *Culture Chambers*. Inexpensive alternatives to CO$_2$ incubator. Upper shelf, custom-made clear plastic box (Courtesy of Reeve Irvine Institute); lower shelf, anaerobic jar (BD Biosciences.)

**Water Bath**

- A device for regulating the temperature of anything subjected to heat, by surrounding the vessel containing it with another vessel containing water which can be kept at a desired temperature;
- tool used to maintain a very stable temperature much like an incubator.
Centrifuge

- A laboratory centrifuge: driven by a motor, which spins liquid samples at high speed.\[\]

Like all other centrifuge, laboratory centrifuges work by the sedimentation principle to separate substances of greater and lesser density.\[\]

- **Centrifugation is used to remove**
  - protein products.
  - dead cells
  - cell debris, etc.

FIGURE 4.18 *Tubes.* Centrifuge and samples tubes, available sterile but non–tissue-culture grade, although tissue culture grade tubes are available (BD Biosciences; Corning). Clockwise from the left: 250-mL centrifuge tube (Corning), 5-mL Bijou bottle (Sterilin), 30-mL universal container (Sterilin), 50-mL centrifuge tube (BD Biosciences), 15-mL centrifuge tube (BD Biosciences), and 5-mL sample tube (BD Biosciences).
Refrigerators

- For small cell culture laboratories, a domestic refrigerator 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.

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.

- While most reagents can withstand temperature oscillations in an autodedefrost freezer

- some reagents such as antibiotics and enzymes should be stored in a freezer that does not autodedefrost.
**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.

- This **liquid nitrogen** method has been successful with many species that cannot be preserved by lyophilization (freeze-drying).

**THIS LIQUID NITROGEN METHOD**

- most species can remain viable under these conditions for 10 to 30 years without undergoing change in their characteristics.

- this method is expensive
Types of liquid nitrogen

1. Vapor phase systems (-196 °C): minimize the risk of explosion with cryostorage tubes, and are required for storing biohazardous materials.

2. Liquid phase systems (-156 °C): usually have longer static holding times, and are therefore more economical.

PH meter

- Is an electronic instrument used for measuring the pH (acidity or alkalinity) of a liquid.

- The pH value of a substance is directly related to the ratio of the hydrogen ion and hydroxyl ion concentrations.

- If the H+ concentration is higher than OH-, the material is acidic.
pipettes

(also called a pipet, pipettor, or chemical dropper) is a laboratory tool used to transport a measured volume of liquid.

Flasks

- Tissue culture flasks provide an even surface refinement in the growth zone.

- The inside surface treatment must provide an optimal growth surface on the flask base.

- The modern tissue culture flasks are available with filtered or vented caps which ensure the accessibility of the interior of the flask, and the cap closure.
FIGURE 4.16 *Bench-Top Autoclave.* Simple, top-loading autoclave from Prestige Medical; left with lid closed, right with lid removed for filling. (Courtesy of Beatson Institute.)
FIGURE 4.17 *Freestanding Autoclave.* Medium-sized (300 L; 10 ft³) laboratory autoclave with square chamber for maximum load. (Courtesy of Beatson Institute.)

**Storage Area**

for liquids: media and reagents
for chemicals: drugs and antibiotics
for consumables: disposable pipettes, culture vessels, and gloves
for glassware: media bottles and glass pipettes
for tissues and cells.

it is important to store all media, reagents, and chemicals according to the instructions on the label.
ASEPTIC TECHNIQUE 1

- Controlled environment
  - Traffic, air flow
- Sterile media and reagents
- Avoids aerial contamination of solutions
- Avoids manual contamination of equipment

ASEPTIC TECHNIQUE 2

- Minimise traffic
- Clear work area
- 70% ethanol swab
- Minimise work area (field of vision)
- Keep work area clean
- Do not lean over open vessels
- UV irradiation before and after
- Only use disposable equipment once
ASEPTIC TECHNIQUE 3

- Minimise exposure to air
- Avoid repeated opening of bottles
- Avoid liquid accumulation around necks and lips of bottles
- Avoid excessive agitation
- Only one cell type at a time
- Do not open contaminated solutions
- No burner in hood

CONTAMINANT’S OF CELL CULTURE

Cell culture contaminants of two types:

Chemical-difficult to detect. caused by endotoxins, plasticizers, metal ions or traces of disinfectants that are invisible

Biological-cause visible effects on the culture they are mycoplasma, yeast, bacteria or fungus or also from cross-contamination of cells from other cell lines
EFFECTS OF BIOLOGICAL CONTAMINATION

They compete for nutrients with host cells
Secreted acidic or alkaline by-products cease the growth of the host cells
Degraded arginine & purine inhibits the synthesis of histone and nucleic acid
They also produce H$_2$O$_2$ which is directly toxic to cells

DETECTION OF CONTAMINANTS

In general: turbid culture media, change in growth rates, abnormally high pH, poor attachment, graining cellular appearance, vacuolization, inclusion bodies and cell lysis

Mycoplasma detected by enzyme immunoassay by specific antisera or monoclonal abs or by PCR amplification of mycoplasmal RNA
CONTAMINATION

- Contamination with other cell lines-
cross contamination
- Yeast
- Fungi
- Viruses:
  - especially bovine Pestiviruses
  - BVDV – Virus of Bovine Virus diarrhea
  - CSFV – Virus of the classical swine
  - but also BDV (Borna Disease Virus)
- Bacteria
- Mycoplasma