Tissue Culture

- **History**
- **Animal Cell Culture**
- **Equipment**
- **Cell Culture Media (培養基)**
- Aseptic Technique
- General Methods of Analysis
- Measurement of Cell Characteristics
- Measurement of Tissue Characteristics

History

- In 1885, Wilhelm Roux maintained tissues from embryonic chicken in warm saline for days, establishing the principle of cell culture.
- Ross G. Harrison published his work from 1907-1910, establishing the methodology of tissue culture.
- In the 1940's and early 1950's, cell culture techniques were advanced significantly, in part, to support research in virology (病毒學). Growing viruses in cell cultures allowed preparation of purified viruses for mass production of vaccines.
  - In 1949, Alan Park developed a protocol of cryopreservation.
  - A famous cell line HeLa cells was derived from cervical cancer (子宮頸癌) cells taken from **He**nrietta **La**cks, who died in 1951.
  - In 1955, Eagle formed the basis of a medium known as "basial medium, Eagle" or "BME", which was replaced by Eagle's minimum essential medium (MEM).

Animal Cell Culture

- Organ Culture
  - A 3-D culture of nondisaggregated tissue or whole organ retaining some or all of the histological features of the tissue in vivo
  - cannot be propagated; greater experimental variation b/w replicates; due to minor variations in geometry and constitution, more difficult to use in quantitative determinations.

Animal Cell Culture

- Cell Culture
  - Derived from dispersed cells taken from the original tissue, from a primary culture, or from a cell line
  - can be purified by growth in selective media, physical cell separation, or by cloning to tive a characterized cell line with uniformity; can be propagated to generate identical replicated; can be cryopreserved
  - lack of structural organization at the tissue level; lost of histotypic architecture and/or biochemical properties associated with the tissue

Adherent Cells vs. Suspension Cells

- Adherent Cells
  - Adherent cells are anchorage-dependent and usually propagate as a monolayer attached to the substrate.
  - The attachment is essential for proliferation
  - Adherent cells stop proliferating once they become confluent (i.e., when they completely cover the surface of the substrate) – a phenomena known as contact inhibition
  - Some cells will die if they are left in the confluent state for too long.

Adherent Cells vs. Suspension Cells

- Suspension Cells
  - Suspension cells can survive and proliferate without being attached to a surface – anchorage independence
  - Hematopoetic cells (derived from blood, spleen, or bone marrow) as well as some transformed cell lines and cells derived from malignant tumors can grow in suspension.

Primary Culture:

A primary culture is that stage of the culture following isolation of the cells, but **before** the first subculture. They are usually more representative of the cell types in the tissue from which they were derived and in the expression of tissue-associated properties.

- From the outgrowth of migrating cells from an explant
- From tissue that is disaggregated mechanically or enzymatically

- Subculture allows the expansion of the culture (now known as a cell line) but may cause a loss of specialized cells and differentiated properties (de-differentiation).

Cell Lines

Once a primary culture is *subcultured* (or *passaged*, or *transferred*), it becomes known as a cell line. This term implies the presence of several cell lineages of either similar or distinct phenotypes.

- To provide large amounts of consistent cells for prolonged used
- Finite cell lines vs. continuous cell lines
  - Cells lines with limited culture lifespans are known as finite cell lines; cells grow through a limited number of cell generations.
  - If a cell line transforms in vitro, it becomes a continuous cell line, which is capable of unlimited proliferation

Continuous Cell Lines

- Greater growth rate; higher cell densities
- Require lower concentrations of serum and are more easily maintained in simple media
- **HOWEVER**, the appearance of a continuous cell line is usually marked by an alteration in cytomophology (smaller cell size, less adherent, more rounded), an increase in heteroploidy (chromosomal variation among cells) and aneuploidy (divergence from the euploid donor karyotype) and a loss of tissue-specific markers.

Euploid: the normal number of chromosomes for a species

TABLE 12.2 Properties of Finite and Continuous Cell Lines

Properties	Finite	Continuous (transformed)
Ploidy	Euploid, diploid	Aneuploid, heteroploid
Transformation	Normal	Immortal, growth-control altered, and tumorigenic
Anchorage dependence	Yes	No
Contact inhibition	Yes	No
Density limitation of cell proliferation	Yes	Reduced or lost
Mode of growth	Monolayer	Monolayer or suspension
Maintenance	Cyclic	Steady state possible
Serum requirement	High	Low
Cloning efficiency	Low	High
Markers	Tissue specific	Chromosomal, enzymic, antigenic
Special functions (e.g., virus susceptibility, differentiation)	May be retained	Often lost
Growth rate	Slow (T_n of 24–96 h)	Rapid (T_n of 12–24 h)
Yield	Low	High
Control parameters	Generation no.; Tissue-specific markers	Stain characteristics

Selection of Cell Line

- Finite vs. continuous
- Normal or Transformed
- Species
- Growth characteristics (population-doubling time, saturation density, cloning efficiency, growth fraction, and ability to grow in suspension)
- Availability
- Validation
- Phenotypic expression
- Control cell line
- Stability

Essential Equipment

- Laminar-flow hood
- CO_2 incubator
- Sterilizer / Autoclave
- Refrigerators and freezers
- Inverted microscope
- Washing up, sterilizing and drying
- Water purification
- Centrifuge
- Cryostorage container
- Balance
- Hemocytometer

Laminar-Flow Hood

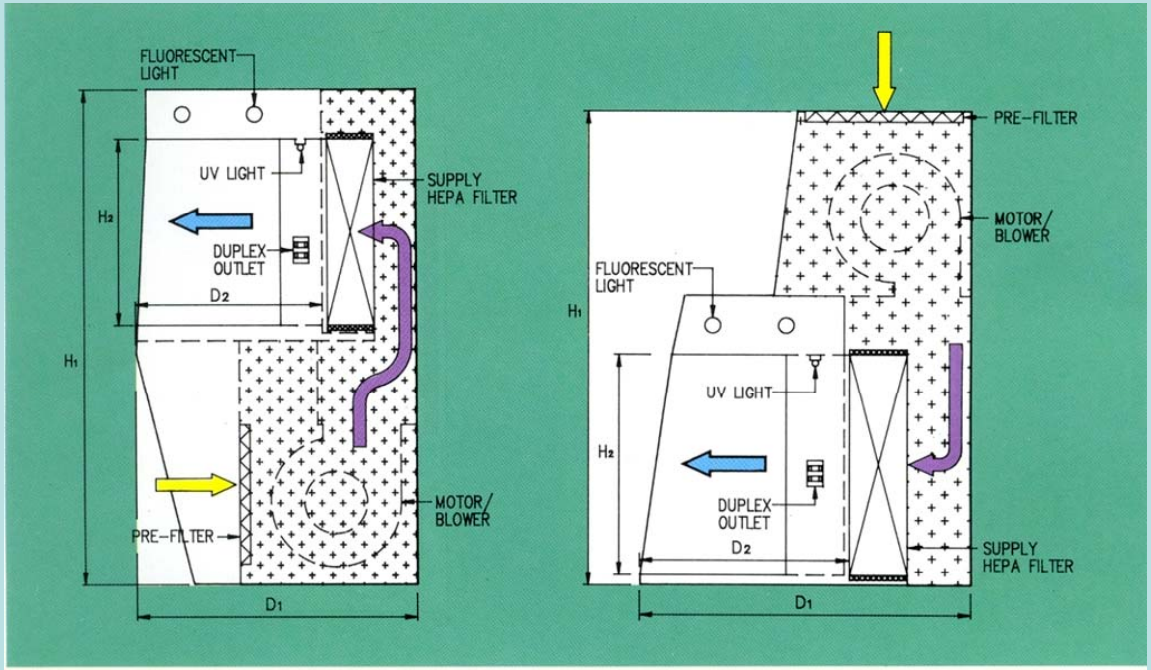
Most tissue culture work is done in a laminar flow hood. The major advantage of this is that the working environment is protected from dust and contamination by a constant, stable flow of filtered air passing over the work surface.

There are two types:

- Horizontal: gives the most stable airflow and best sterile protection to the culture and reagents
- Vertical: gives more protection to the operators

If potentially hazardous materials (radioisotopes, human- or primate-derived cultures, virus-infected cultures, etc.) are being handled, a Class II biological safety cabinet should be used. If known human pathogens are handled, a Class III biological safety cabinet is obligatory.

Horizontal Laminar-Flow Hood



規範說明：

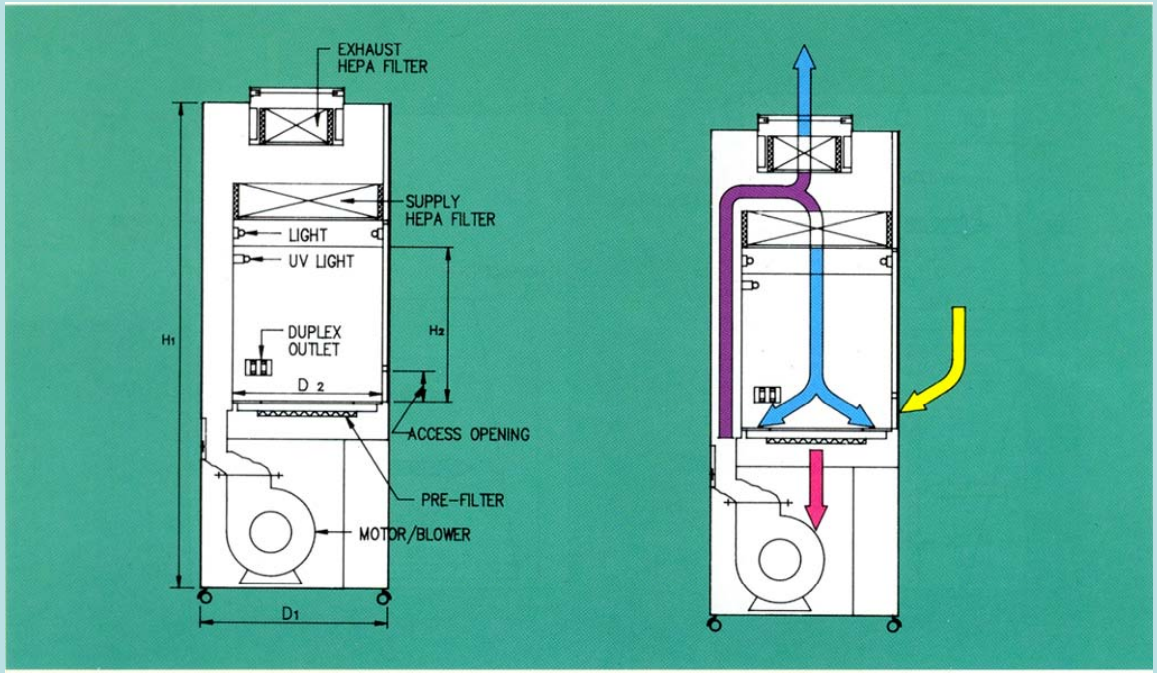
1. 主體結構：冷作鐵板烤漆及不銹鋼製工作台面。
2. 高效率過濾網(HEPA FILTER)：D.O.P.測試可過濾0.3 MICRON以上之微塵效率99.97％以上。
3. 預濾網(PRE-FILTER)：採用進口V型初級濾網。
4. 風扇馬達(MOTOR／BLOWER)：美製原裝進口、低噪音、低震動。
5. 平均風速：100+－20 F.P.M。
6. 標準配件：①計時器 ②電源雙插座 ③紫外線殺菌燈。

ROOM AIR

DIRTY AIR POSITIVE PRESSURE

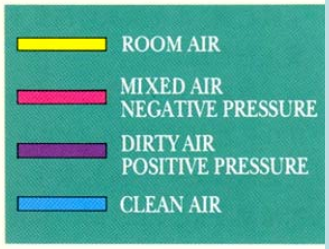
CLEAN AIR

Class II Biological Safety Cabinet



規範說明：

1. 主體結構外箱：冷作鐵板烤漆
   正面及內部操作範圍：不銹鋼 304
   一體成型製作。
2. 高效率過濾網（HEPA FILTER）：
   D.O.P測試可過濾 0.3 MICRON以
   上之微塵效率99.97％以上。
3. 預濾網（PRE-FILTER）：採用進口V
   型初級濾網。
4. 風扇馬達（MOTOR/BLOWER）：美
   製原裝進口、低噪音、低震動。
5. 滑動拉門可配合操作需要，隨意調
   整其高度，視窗採 5 ㎜厚，雙色玻璃
   ，能阻隔紫外殺菌燈光線。
6. 平均風速：100＋－20F.P.M.。
7. 標準配件：①計時器 ②氣體開關
   ③紫外線殺菌燈 ④電源雙插座



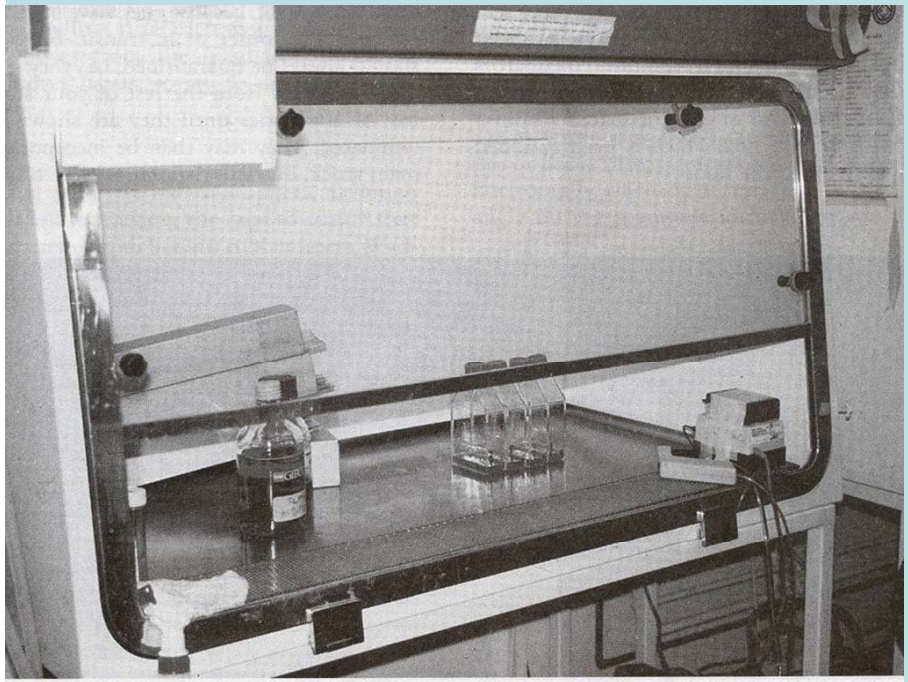


*Fig. 5.2. **Suggested Layout of Work Area.*** Laminar-flow hood laid out correctly. Positions may be reversed for left-handed workers.

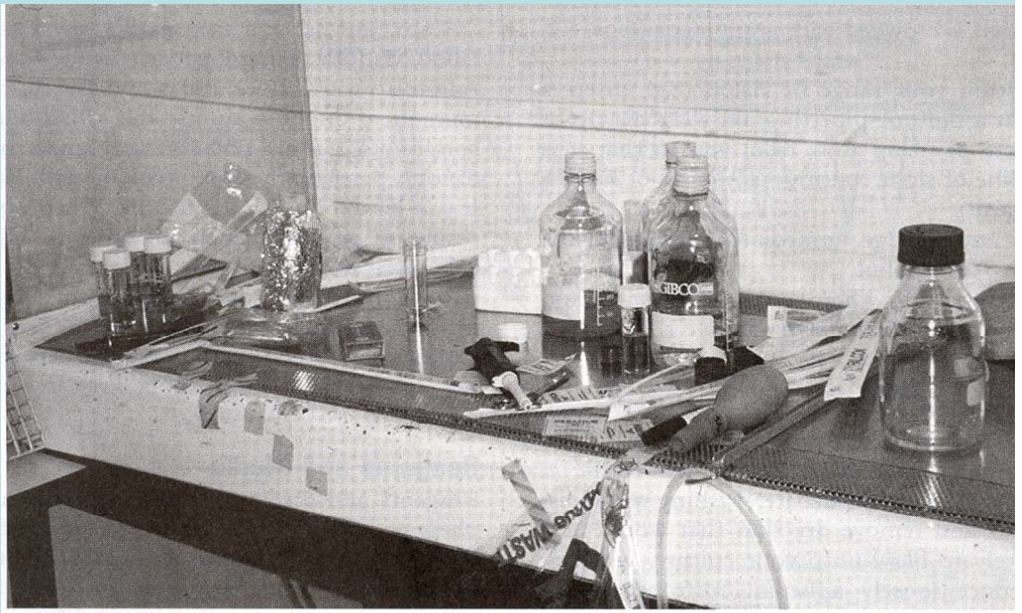


*Fig. 5.3. **Badly Arranged Work Area.*** Laminar-flow hood being used incorrectly. The hood is too full, and many items encroach on the air intake at the front, destroying the laminar airflow and compromising both containment and sterility.

CO_2 Incubator

Some vessels, e.g., Petri dishes or multi-well plates, require a controlled atmosphere with high humidity and elevated CO_2 tension.

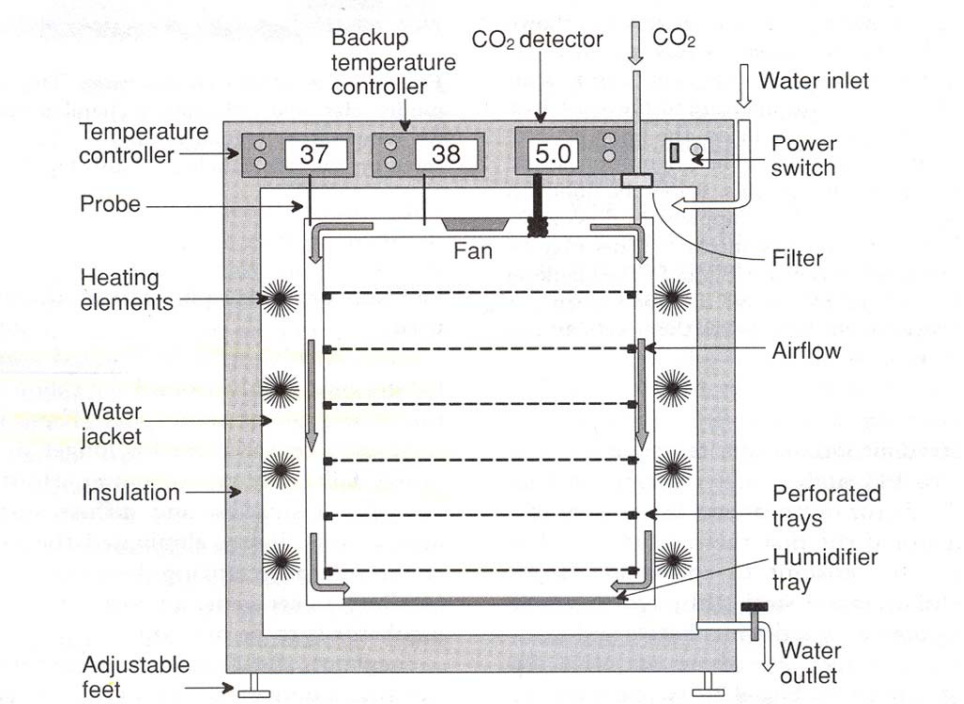


Fig. 4.3. CO_2 Incubator Design. Front view of control panel and section of chamber of a stylized humid CO_2 incubator (not representative of any particular make).

Washing and Sterilizing Glassware

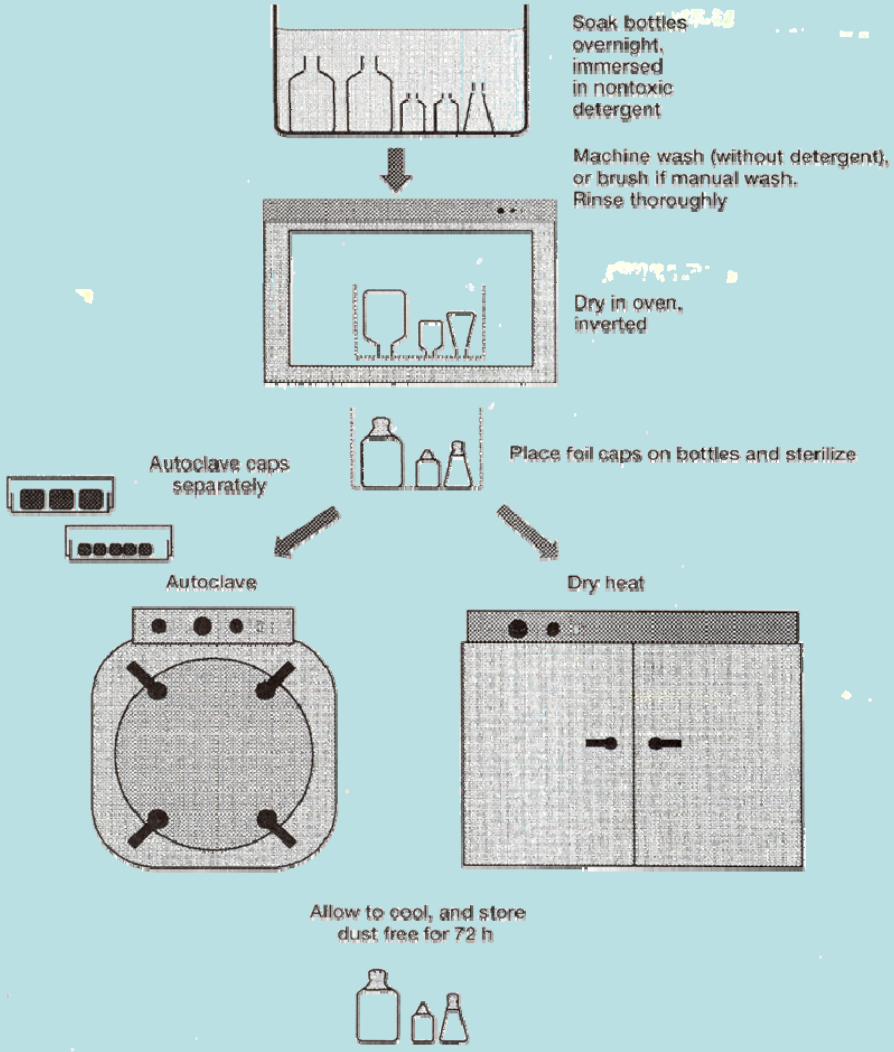


Fig. 10.1. Washing and Sterilizing Glassware. Procedure as in Protocol 10.1. Sterilization conditions: autoclave at 121°C for 15 min; oven, 160°C for 1 h. Caps are sterilized separately from bottles to avoid condensation forming in bottles if autoclaved with caps in place. (See Fig. 10.3.)

Sterilizer / Autoclave

Filtration

Fig. 10.12. Sterilizing Filters. Disposable filters. a. Millex 25-mm disc syringe filter (Millipore). b. Sterivex in use with repeating syringe. c. Bottle-top fitting (Becton-Dickinson). d. Filter cup and storage vessels (Stericup, Millipore). (a,b) positive pressure; (c,d) negative pressure (see also Figure 10.11). Photographs a, b, and d courtesy of Millipore (UK), Ltd.

Inverted Microscope

A morphological change is often the first sign of deterioration in a culture and the characteristic pattern of microbiological infection is easily recognized.

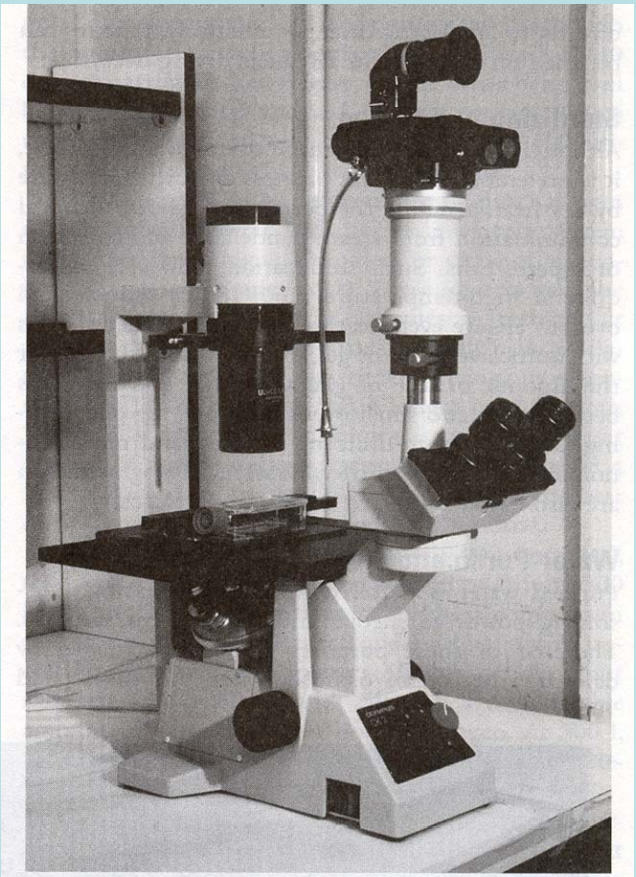


Fig. 4.6. Inverted Microscope. Olympus CK2 inverted microscope fitted with phase-contrast optics and trinocular head with automatic camera.

Upright microscope

Water Purification System

Purified water is required for rinsing glassware, dissolving powdered media, and diluting concentrates.

Fig. 4.7. Preparation of Ultrapure Water. Tap water first passes through a reverse-osmosis unit on the right and then goes to the storage tank on the left. It then passes through carbon filtration and deionization (center unit) before being collected via a micropore filter (Millipore MillQ).

Cell Culture Media

When a cell is removed from its original tissue and placed in culture, the medium must provide all the environmental conditions (i.e., nutritional, hormonal, and stromal) that the cell has been exposed to in vivo.

- Biological buffers
- Serum (plasma without fibrinogen and other clotting factors)
- Antibiotics

Biological Buffers

Known as "physiological" or balanced salt solution

The function of this salt solution in the media are to

- maintain proper pH (7.2-7.4)
  - Sodium bicarbonate
    - pKa=6.3 at 37°C
    - required atmospheric CO_2
  - HEPES
    - expensive
- maintain ideal osmotic pressure (260-320 mosm)
- provide a source of energy, e.g., glucose

Serum

Among the biological fluids that have proved successful for culturing cells, serum is the most popular. Generally, 5-20% serum is usually needed for optimal cell growth in culture.

Some of the major functions of serum are to provide

- Basic nutrients (both in solution and bound to proteins)
- Hormones and growth factors, e.g., insulin
- Attachment and spreading factors, e.g., fibronectin
- Binding proteins (albumin, transferrin) for carrying hormones, vitamins, lipids, etc.
- Non-specific protection factors against mechanical damage – provide viscosity to reduce shear forces during agitation of cell suspension
- Protease inhibitors
- pH buffering

Disadvantages of Using Serum in Cell Culture

- For most cells, serum is not the physiological fluid that they come in contact with in the original tissue except during wound healing and blood clotting.
- Serum can sometimes be cytotoxic. It can contain bacterial toxins and other inhibitors.
- Batch-to-batch variations in serum can necessitate time-consuming and costly serum screening.
- Serum may contain inadequate amounts of some growth factors and overabundance of others
- Serum provides a risk of contamination.
- Sterilization problems associated with filtration of colloids and particulate content.

Advantages of Low-Serum or Serum-Free Medium

- Improved reproducibility between cultures and avoidance of batch-to-batch variations
- Standardization of media formulations among different laboratories
- Improved economy
- Easier purification of culture products
- Less protein interference in bioassays
- Avoidance of serum cytotoxity
- No serum proteases to degrade sensitive proteins
- Selective culture of differentiated and functional cell types from heterogeneous populations of primary cultures