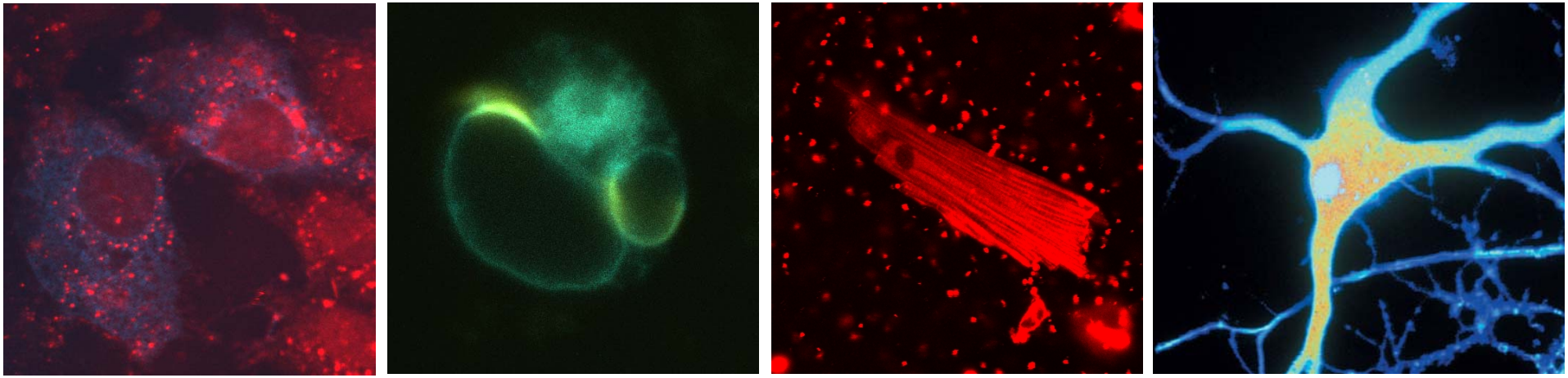
Cell culture techniques



Cell culture

Advantages

- Study of cell behaviour without the variations that occur in animal
- Control of the growth environment leads to uniformity of sample
- Characteristics of cells can be maintained over several generations, leading to good reproducibility between experiments

Cell culture

Advantages

- Cultures can be exposed to reagents e.g. radio-chemicals or drugs at defined concentrations
- Finally it avoids the legal, moral and ethical problems of animal experimentation

Cell culture

Disadvantages

- Have to develop standardised techniques in order to maintain healthy reproducible cells for experiments
- Takes time to learn aseptic technique
- Quantity of material is limited
- Dedifferentiation and selection can occur and many of the original cellular mechanisms can be lost

Cell culture

Terminology

Organ Culture

- A three dimensional culture of undisaggregated tissue retaining some or all of the features of the tissue in vivo

Cell Culture

- Single cells, no longer organised as tissues. Derived from dispersed cells taken from the original tissue

Primary Cell Culture

- Derived from an explant, directly from the animal
- Usually only survive for a finite period of time
- Involves enzymatic and/or mechanical disruption of the tissue and some selection steps to isolate the cells of interest from a heterogeneous population

Cell culture

Terminology

Clone

- A population derived from a single cell

Sub-culture

- Transplantation of cells from one vessel to another

Established or Continuous Cell Lines

- A primary culture that has become immortal due to some transformation
- Most commonly tumour derived, or transformed with a virus such as Epstein-Barr
- One of the most commonly used cells are Chinese Hamster Ovary cells (CHO)
- The SH-SY-5Y cells a human neuroblastoma derived cell line

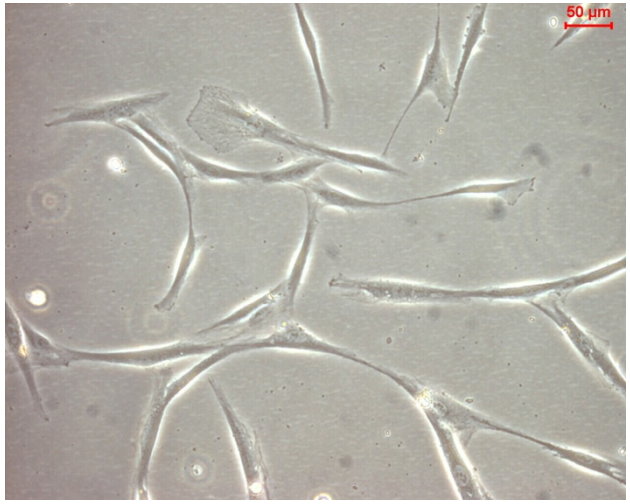
Passage Number

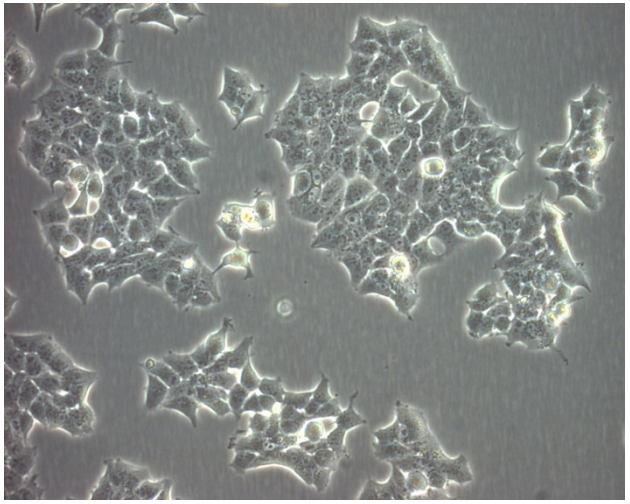
- Number of successive sub-cultures from primary culture

Cell culture

Morphology

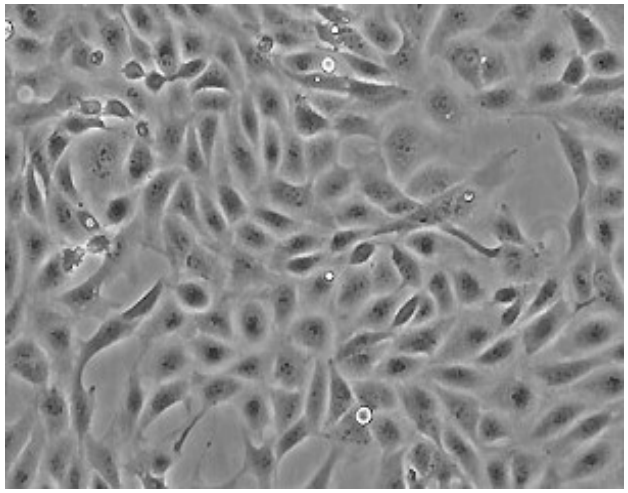
Fibroblastic

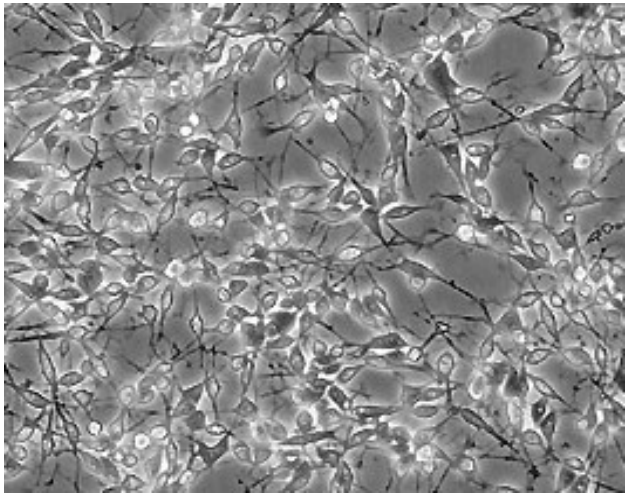




Epithelial

Endothelial





Neuronal

Cell culture

Cell culture media

What do cells need to grow?

- **Substrate or liquid (cell culture flask or scaffold material)**
  - **chemically modified plastic or coated with ECM proteins**
  - **suspension culture**
- **Nutrients (culture media)**
- **Environment (CO_2, temperature 37^{o}C, humidity)**
  - **Oxygen tension maintained at atmospheric but can be varied**
- **Sterility (aseptic technique, antibiotics and antimycotics)**
  - **Mycoplasma tested**

Cell culture

Cell culture media

Basal Media

- Maintain pH and osmolarity (260-320 mOsm/L).
- Provide nutrients and energy source.

Components of Basal Media

Inorganic Salts

- Maintain osmolarity
- Regulate membrane potential (Na^+, K^+, Ca^{2+})
- Ions for cell attachment and enzyme cofactors

pH Indicator – Phenol Red

- Optimum cell growth approx. pH 7.4

Buffers (Bicarbonate and HEPES)

- Bicarbonate buffered media requires CO_2 atmosphere
- HEPES Strong chemical buffer range pH 7.2 – 7.6 (does not require CO_2)

Glucose

- Energy Source

Cell culture

Cell culture media

Components of Basal Media

Keto acids (oxalacetate and pyruvate)

- Intermediate in Glycolysis/Krebs cycle
- Keto acids added to the media as additional energy source
- Maintain maximum cell metabolism

Carbohydrates

- Energy source
- Glucose and galactose
- Low (1 g/L) and high (4.5 g/L) concentrations of sugars in basal media

Vitamins

- Precursors for numerous co-factors
- B group vitamins necessary for cell growth and proliferation
- Common vitamins found in basal media is riboflavin, thiamine and biotin

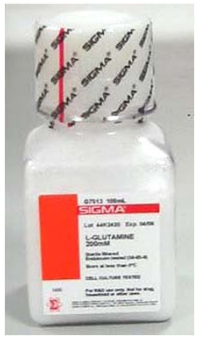
Trace Elements

- Zinc, copper, selenium and tricarboxylic acid intermediates

Cell culture

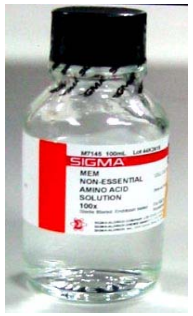
Cell culture media

Supplements



L-glutamine

- Essential amino acid (not synthesised by the cell)
- Energy source (citric acid cycle), used in protein synthesis
- Unstable in liquid media - added as a supplement



Non-essential amino acids (NEAA)

- Usually added to basic media compositions
- Energy source, used in protein synthesis
- May reduce metabolic burden on cells

Growth Factors and Hormones (e.g.: insulin)

- Stimulate glucose transport and utilisation
- Uptake of amino acids
- Maintenance of differentiation

Antibiotics and Antimycotics

- Penicillin, streptomycin, gentamicin, amphotericin B
- Reduce the risk of bacterial and fungal contamination
- Cells can become antibiotic resistant – changing phenotype
- Preferably avoided in long term culture

Cell culture

Cell culture media

Foetal Calf/Bovine Serum (FCS & FBS)

- Growth factors and hormones
- Aids cell attachment
- Binds and neutralise toxins
- Long history of use

- Infectious agents (prions)
- Variable composition
- Expensive
- Regulatory issues (to minimise risk)

Heat Inactivation (56°C for 30 mins) – why?

- Destruction of complement and immunoglobulins
- Destruction of some viruses (also gamma irradiated serum)

Care! Overdoing it can damage growth factors, hormones & vitamins and affect cell growth

Cell culture

Cell culture media

Revive frozen cell population
Isolate from tissue

⇩

Maintain in culture (aseptic technique)

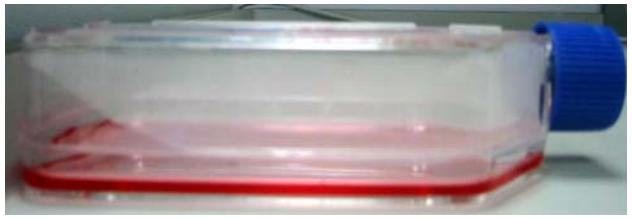
⇩

Sub-culture (passaging)

⇩ **Count cells**

Cryopreservation

Containment level 2 cell culture laboratory



Typical cell culture flask

'Mr Frosty' Used to freeze cells

Cell culture

How to keep the cells alive

Check confluency of cells

↓

Remove spent medium

↓

Wash with PBS

↓

Incubate with trypsin/EDTA

↓

Resuspend in serum containing media

↓

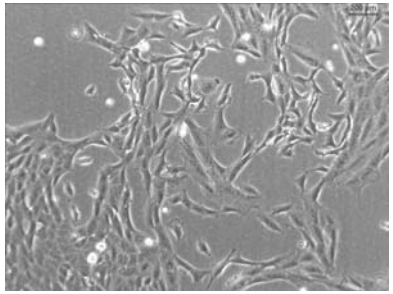
Transfer to culture flask

Why passage cells?

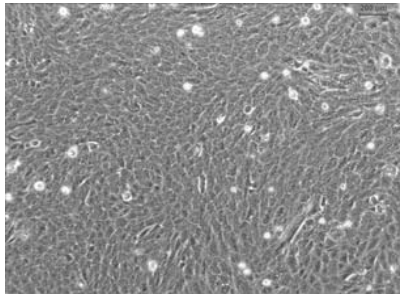
- To maintain cells in culture (i.e. don't overgrow)
- To increase cell number for experiments/storage

How?

- 70-80% confluency
- Wash in PBS to remove dead cells and serum
- Trypsin digests protein-surface interaction to release cells (collagenase also useful)
- EDTA enhances trypsin activity
- Resuspend in serum (inactivates trypsin)
- Transfer dilute cell suspension to new flask (fresh media)
- Most cell lines will adhere in approx. 3-4 hours



70-80% confluence



100% confluence

Cell culture

Cryopreservation

Passage cells

⇩

Resuspend cells in serum containing media

⇩

Centrifuge & Aspirate supernatant

⇩

Resuspend cells in 10% DMSO in FCS

⇩

Transfer to cryovial Freeze at -80°C

⇩

Transfer to liquid nitrogen storage tank

Why cryopreserve cells?

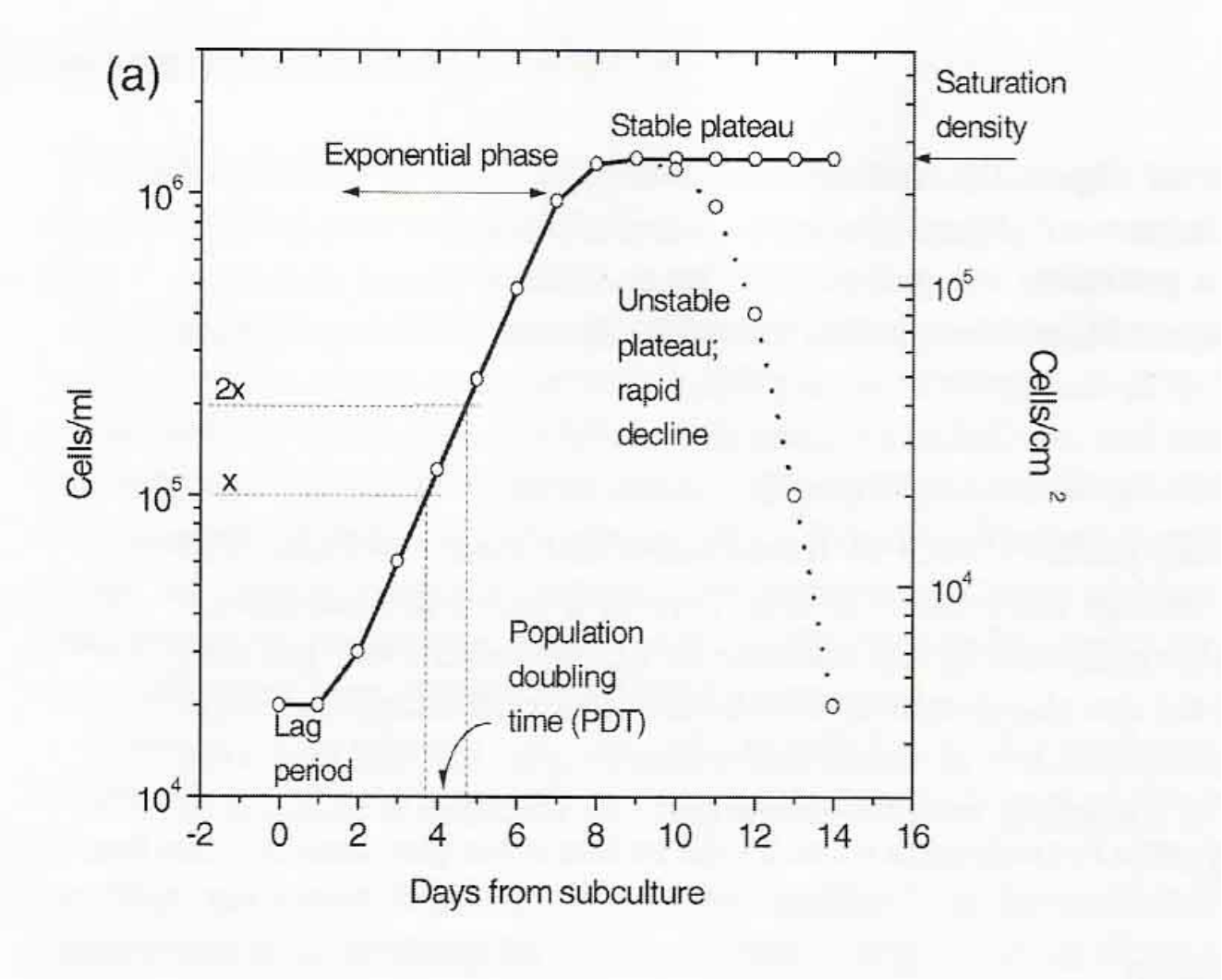
- Reduced risk of microbial contamination.
- Reduced risk of cross contamination with other cell lines.
- Reduced risk of genetic drift and morphological changes.
- Research conducted using cells at consistent low passage.

How?

- Log phase of growth and >90% viability
- Passage cells & pellet for media exchange
- Cryopreservant (DMSO) – precise mechanism unknown but prevents ice crystal formation
- Freeze at -80°C – rapid yet 'slow' freezing
- Liquid nitrogen -196°C

Cell culture

Cell culture media

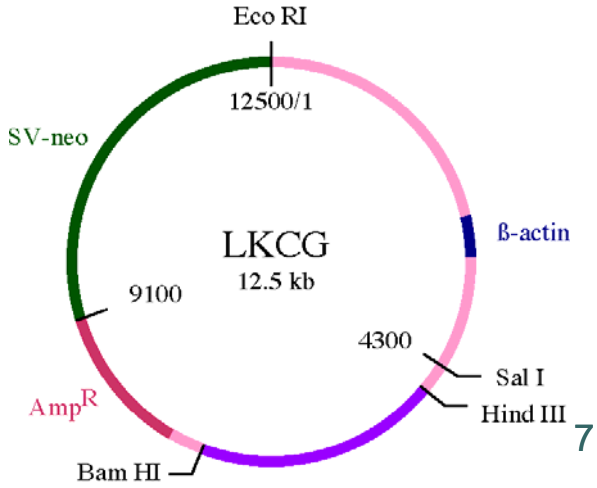


Cell culture

Transfection

Transfection describes the introduction of foreign material into eukaryotic cells using a virus vector or other means of transfer. The term transfection for non-viral methods is most often used in reference to mammalian cells, while the term transformation is preferred to describe non-viral DNA transfer in bacteria and non-animal eukaryotic cells such as fungi, algae and plants.

Transfection of animal cells typically involves opening transient pores or 'holes' in the cell plasma membrane, to allow the uptake of material. Genetic material (such as supercoiled plasmid DNA or siRNA constructs), or even proteins such as antibodies, may be transfected. In addition to electroporation, transfection can be carried out by mixing a cationic lipid with the material to produce liposomes, which fuse with the cell plasma membrane and deposit their cargo inside.



Cell culture

Cell culture enemies

Micro-organisms grow ~10-50 times faster than mammalian cells, which take ~8-16 hours to divide. They are more tolerant to variations in temperature, pH and nutrient supply than cells.

Cells are most vulnerable to contamination when our aseptic technique is bad and the culture becomes infected with bugs.

This can lead to the development of antibiotic resistant micro-organisms.

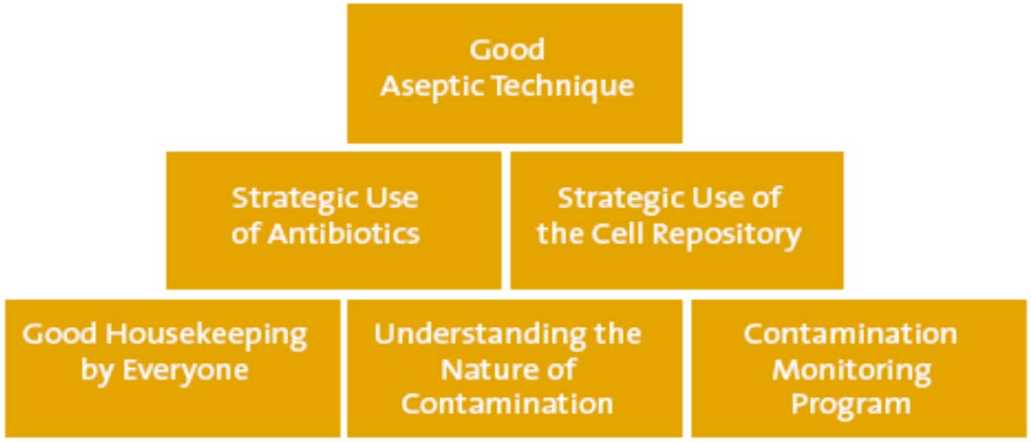
Cell culture

Cell culture enemies

1-Chemical Contamination
Media
Incubator
Serum
water

2-Biological Contamination
Bacteria and yeast
Viruses
Mycoplasmas
Cross-contamination by other cell culture

How Can Cell Culture Contamination Be Controlled?



Cell culture

Safety in Cell Culture

Substances Hazardous to Health

Carcinogen

- A substance that can cause Cancer

Teratogen

- A substance that can cause damage to the developing Foetus

Mutagen

- A substance that can cause a mutation in the genetic material that can be passed to the next generation

Gentamycin and Thapsigargin	Possible Teratogens
Hygromycin	Possible Carcinogen
Streptomycin	Mutagen

Cell culture

Safety in Cell Culture

Waste Disposal

- All waste that has come into contact with cells has to be autoclaved
- Pipettes, flasks, other containers and gloves go into autoclave bags in the bin at the side of the cabinet. Do not leave liquids in these
- Liquid waste goes into the bottles on the trolley in the cell culture suite to be autoclaved. These bottles contain a Chlorine based disinfectant
- Do not overfill waste containers as this causes problems in the autoclave
- Paper waste such as pipette and flask wrappers should go into the black bag lined waste bins

Cell culture

Safety in Cell Culture

Use of Cell Culture areas

- The cell culture area, as any other laboratory is a working area

- Do not bring your friends in with you
- Do not eat, drink or smoke in these areas
- Do not use a mobile phone

- Do wear a lab coat at all times whether in a cell culture area or a laboratory
- Do wear disposable gloves, but make sure that you dispose of them in the correct way before you leave the area

- Do not wear disposable gloves in the corridors or write-up areas

Cell culture

Safety in Cell Culture

Horizontal Laminar Flow Cabinets

- These provide the most sterile environment for the cells, but offer no protection to the operator
- Filtered air enters at the back of the cabinet and is directed to the front, directly at the operator
- The most sterile part of the cabinet is at the back

Cell culture

Safety in Cell Culture

Class II Cabinets

- These cabinets are designed to give operator protection as well as a sterile environment
- The air is directed downwards from the top of the cabinet to the base, when working in these cabinets it is important not to pas non-sterile objects over sterile ones
- Because air is also drawn in from the front of the cabinet, this area is not sterile
- We maintain all our cell lines in class II containment
- Most work with Human or Primate cells must be done in Class II containment

Cell culture

Safety in Cell Culture

Centrifuges

- There are centrifuges in each cell culture area which are refrigerated
- Human derived cells must be centrifuges in sealed rotors
- 100 x g is hard enough to sediment cells, higher g forces may damage cells
- If a tube breaks in the centrifuge, take the whole bucket into a cabinet and clean it there

Cell culture

Safety in Cell Culture

Incubators

- The incubators run at 37C and 5% Carbon Dioxide to keep the medium at the correct pH
- They all have meters on them to register temperature and gas level
- There are alarms to indicate when these deviate from set parameters
- Keep the door open for as short a time as possible

Cell culture

Cell culture enemies

IF YOU ARE IN DOUBT ABOUT THE CONDITION OF YOUR CELLS, ASK FOR ADVICE

NEVER USE CONTAMINATED CELLS. THEY MAY NOT REACT IN THE SAME WAY AS UNCONTAMINATED CELLS

POOR ASEPTIC TECHNIQUE IS THE MAJOR CAUSE OF INFECTIONS