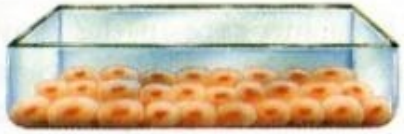
# Tissue Engineering

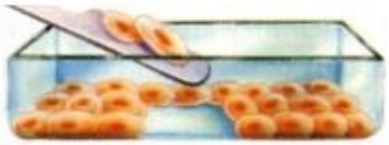
## Course 7

## Cell Culture Equipments

# Demonstrating Contact Inhibition



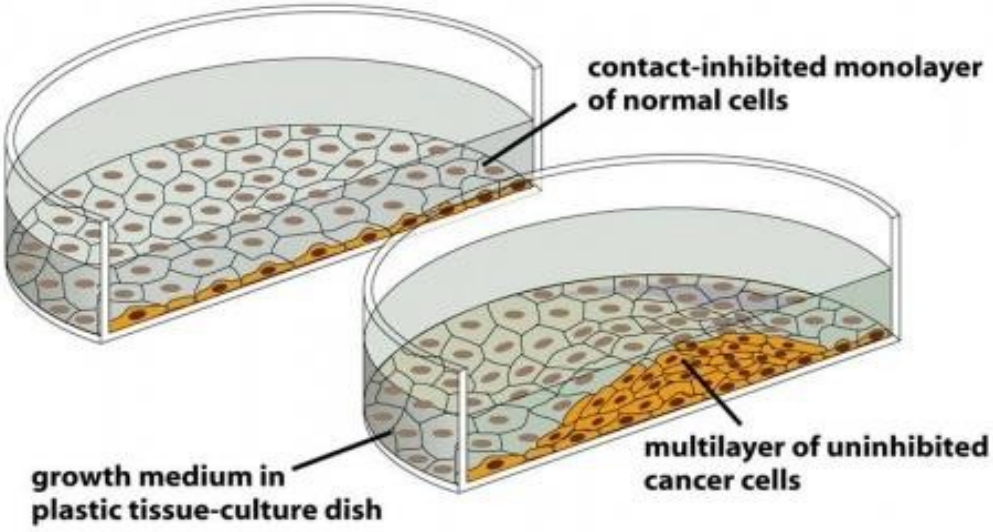
Normal cells in dish



When cells are removed, cells at the edge of gap begin to divide

Normal cells stop dividing when bottom of dish is covered.

Cancer cells continue to divide, piling on top of each other




Focus

# COMMON CELL LINES

**Human cell lines**

- MCF-7 : breast cancer
- HeLa : Henrietta lacks cells

**Mammalian cell lines**

- Vero: African green monkey kidney (epithelial cells)
- BHK: Baby Hamster Kidney cells (fibroblast cells)
- MDCK: Mardin Darby Chicken Kidney cells

**Insect cell lines**

- C636: *Aedes albopictus* (mosquito cells line)

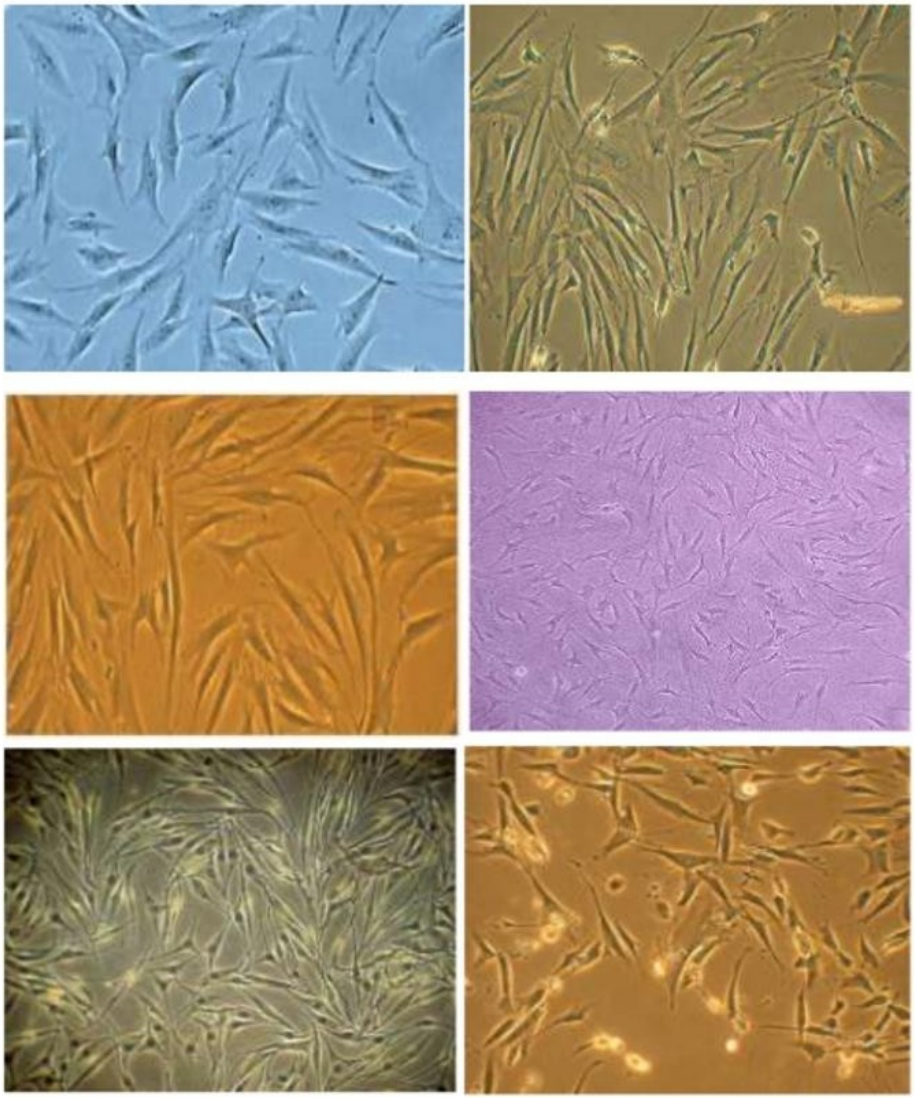
# Morphology of Cells in Culture

❖ **Based on shape and appearance: 3 types**

- **Fibroblast-like**
- **Epithelial-like**
- **Lymphoblast-like**

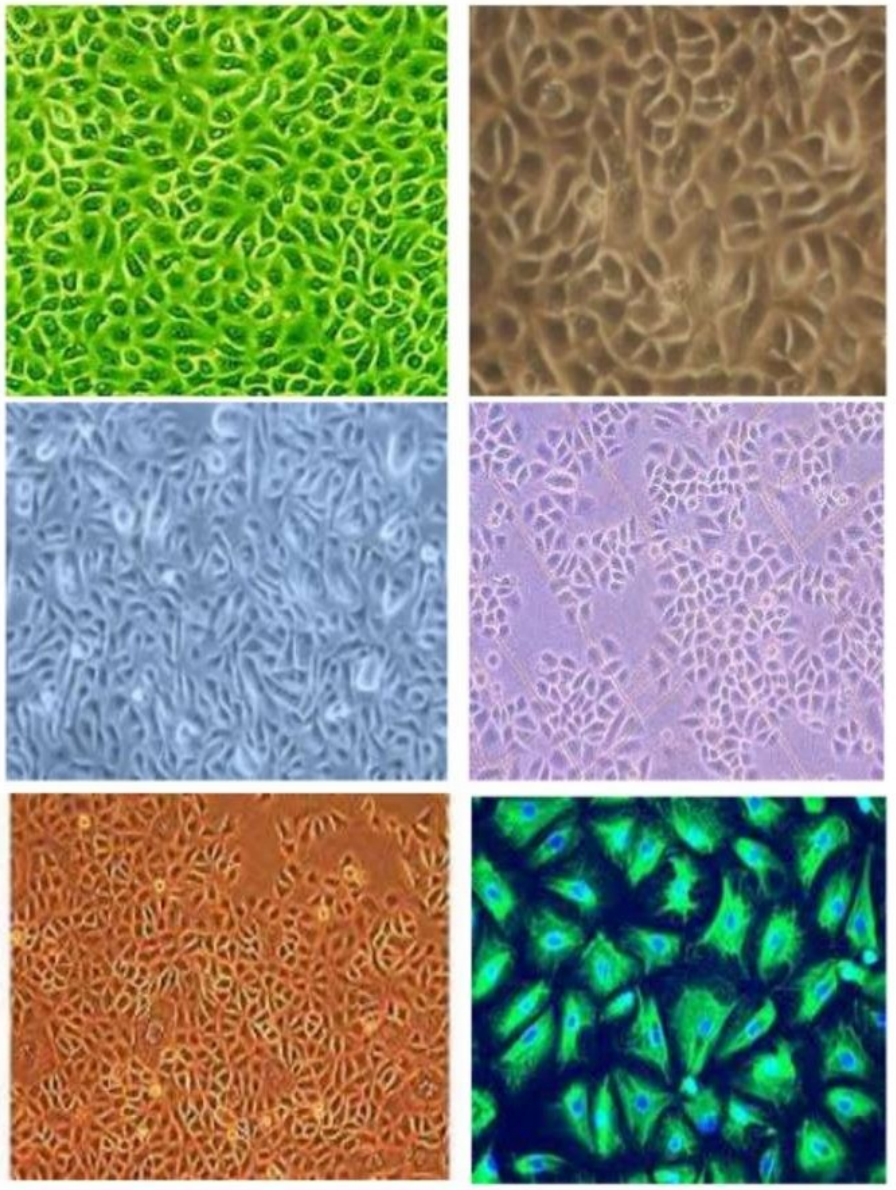
# Morphology of Cells in Culture

- Fibroblast-like cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate



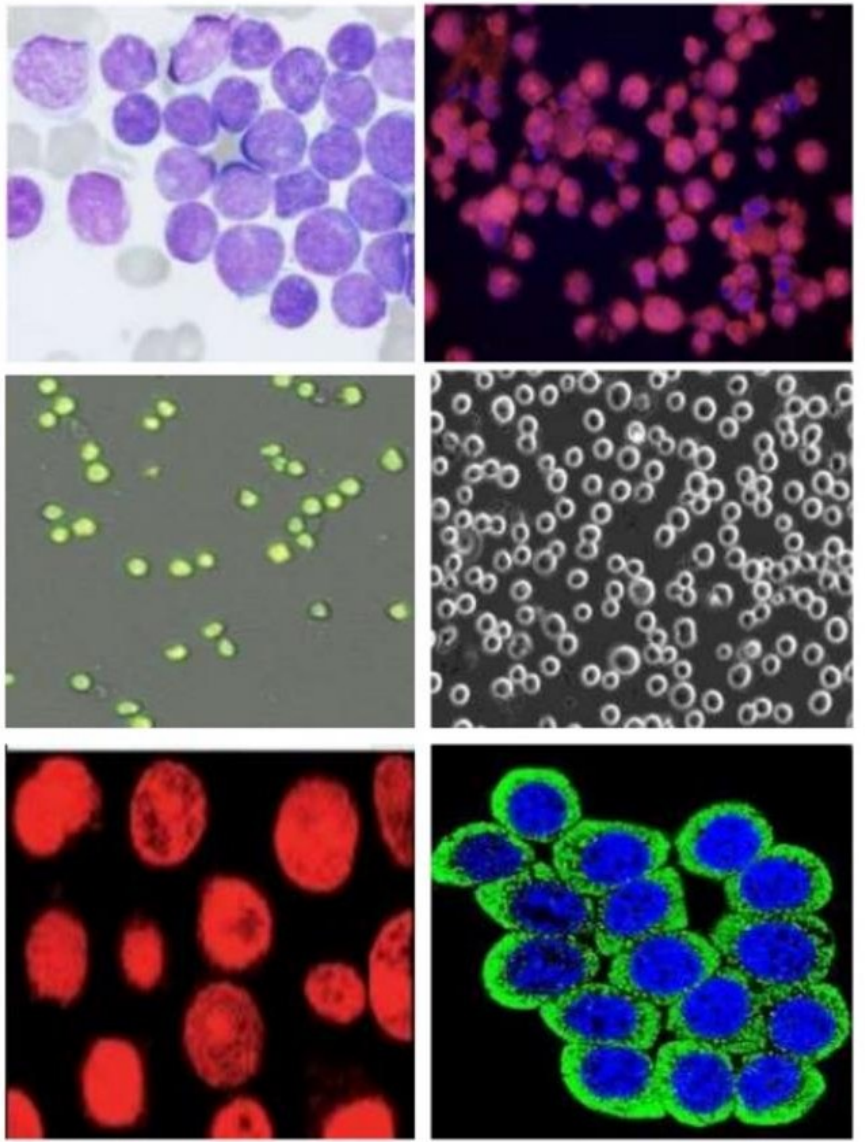
# Morphology of Cells in Culture

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



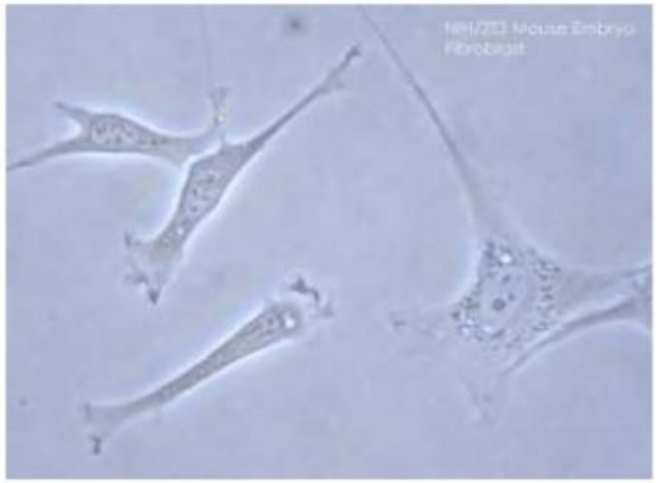
# Morphology of Cells in Culture

- **Lymphoblast-like** cells are spherical in shape and usually grown in suspension without attaching to a surface.



# Morphology of Cells in Culture

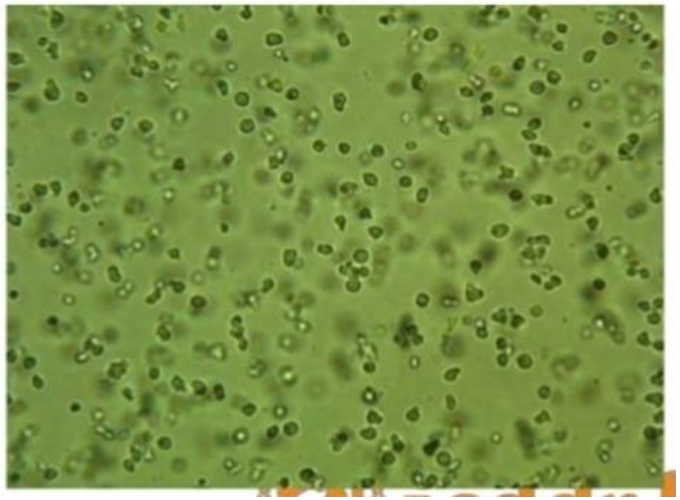
## Depending on the adherence property : 2 types



### Anchorage-dependent

Must be cultured while attached to a solid or semi-solid substrate (**adherent** or **monolayer culture**)

Example: MDCK, Vero



### Anchorage-Independent (**suspension culture**)

Can be grown floating in the culture medium

Example: MNFS-60

# Culturing of cells

- Cells are cultured as anchorage dependent or independent
- Cell lines derived from normal tissues are considered as anchorage-dependent grows only on a suitable substrate e.g. tissue cells
- Suspension cells are anchorage-independent e.g. blood cells
- Transformed cell lines either grows as monolayer or as suspension

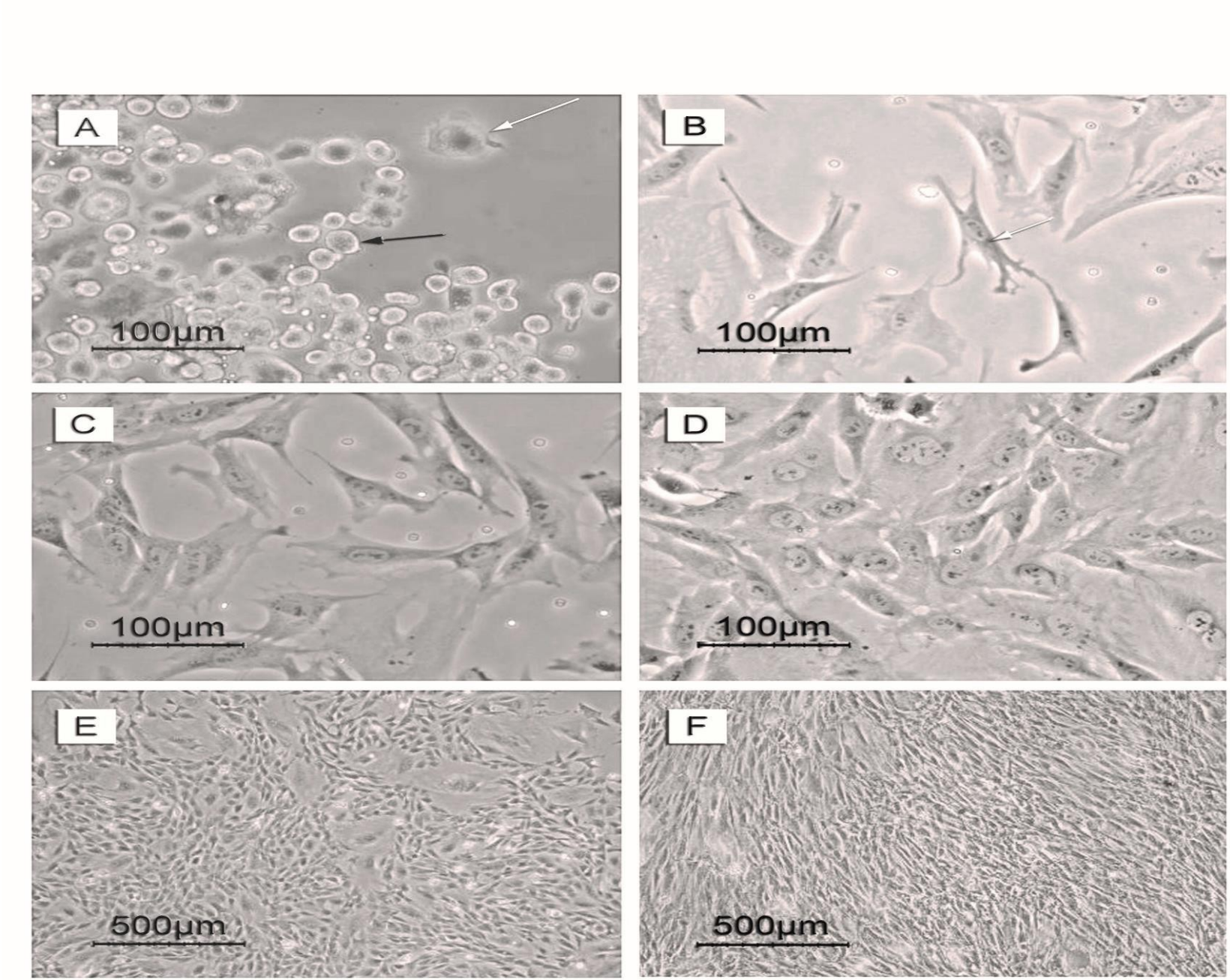
# Adherent cells

- Cells which are anchorage dependent
- Cells are washed with PBS (free of ca & mg ) solution.
- Add enough trypsin/EDTA to cover the monolayer
- Incubate the plate at 37 C for 1-2 mts
- Tap the vessel from the sides to dislodge the cells
- Add complete medium to dissociate and dislodge the cells
- with the help of pipette which are remained to be adherent
- Add complete medium depends on the subculture
- requirement either to 75 cm or 175 cm flask

# Suspension cells

- Easier to passage as no need to detach them
- As the suspension cells reach to confluency
- Asceptically remove 1/3$^{rd}$ of medium
- Replaced with the same amount of pre-warmed medium

# Cell Confluency



# Passaging Cells

Check confluency of cells

⇩

Remove spent medium

⇩

Wash with PBS

⇩

Incubate with trypsin/EDTA

⇩

Resuspend in serum containing media

⇩

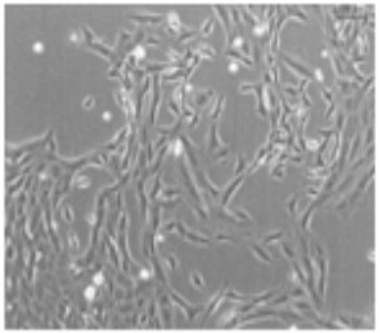
Transfer to culture flask

## Why passage cells?

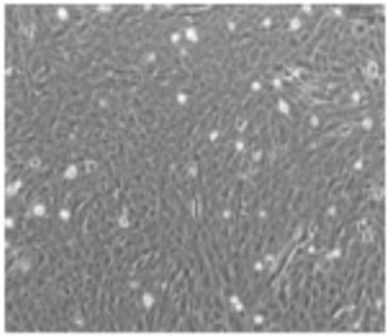
- To maintain cells in culture (i.e. don't overgrow)
- To increase cell number for experiments/storage

## 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

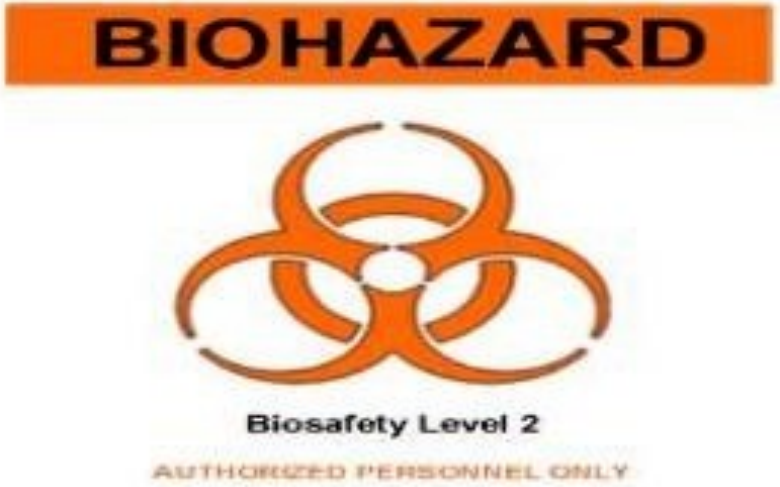


100% confluence

# Cell culture room

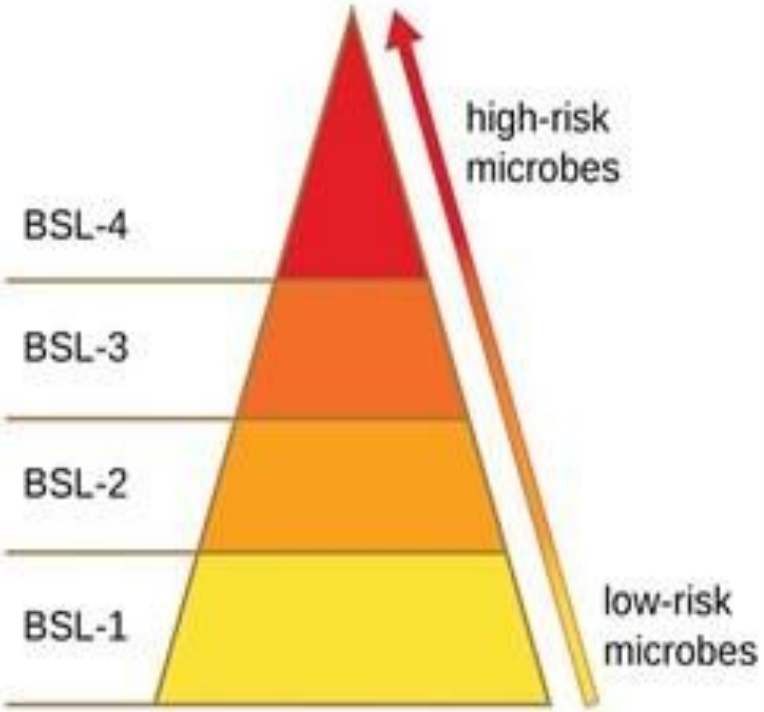


Biosafety is the application of safety precautions that reduce a laboratorians risk of exposure to a potentially infectious material and limit contamination of the work environment and ultimately the community { CDC }



# Why we need biosafety ????

1. Lab has hazards of processing infectious agents
2. Accidental threat to workers and environment
3. To have adherence with safety regulations while dealing with highly infectious agents

| Biosafety Levels | | | |
|---|---|---|---|
| **Biological Safety Levels** | **Description** | **Examples** | **CDC Classification** |
| BSL-4 | Microbes are dangerous and exotic, posing a high risk of aerosol-transmitted infections, which are frequently fatal without treatment or vaccines. Few labs are at this level. | Ebola and Marburg viruses |  |
| BSL-3 | Microbes are indigenous or exotic and cause serious or potentially lethal diseases through respiratory transmission. | *Mycobacterium tuberculosis* | |
| BSL-2 | Microbes are typically indigenous and are associated with diseases of varying severity. They pose moderate risk to workers and the environment. | *Staphylococcus aureus* | |
| BSL-1 | Microbes are not known to cause disease in healthy hosts and pose minimal risk to workers and the environment. | Nonpathogenic strains of *Escherichia coli* | |

# Biosafety Level 1 (BSL-1)

- BSL-1 is the basic level of protection common to most research and clinical laboratories, and is appropriate **for agents that are not known to cause disease in normal and healthy humans.**

# Biosafety Level 2 (BSL-2)

- BSL-2 is appropriate for moderate-risk **agents known to cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure.**
- **Most cell culture labs should be at least BSL-2**, but the exact requirements depend upon the cell line used and the type of work conducted.

# Biosafety Level 3 (BSL-3)

- BSL-3 is appropriate for indigenous or exotic **agents with a known potential for aerosol transmission, and for agents that may cause serious and potentially lethal infections.**

# Biosafety Level 4 (BSL-4)

- BSL-4 is appropriate for exotic **agents that pose a high individual risk of life-threatening disease by infectious aerosols and for which no treatment is available.**
- These agents are restricted to high containment laboratories.

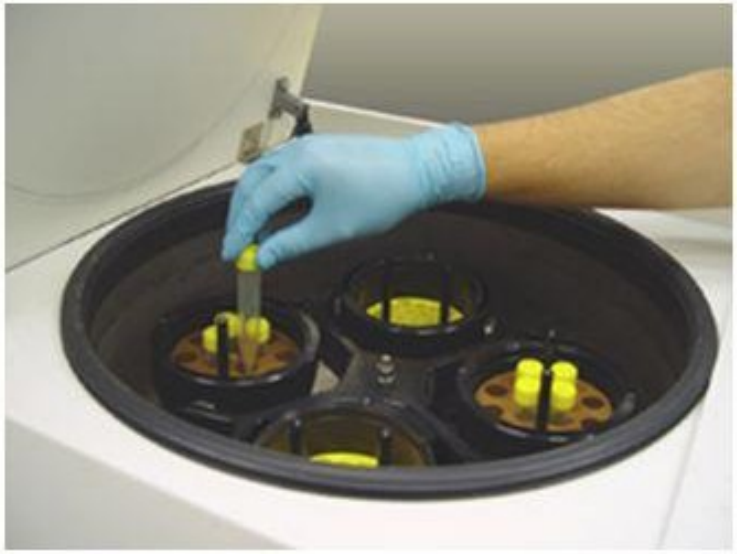

A

# BSL 4 - practices

- Change clothes before entering
- Shower upon exiting
- Decontaminate all materials before exiting
- Class III BSC
- Separate building for lab
- Vacuum lines and decontamination systems

# Equipment

- Cell culture hood
- Incubator
- Water bath
- Centrifuge
- Refrigerator and freezer (–20°C)
- Cell counter (e.g. Automated Cell Counter or hemacytometer)
- Inverted microscope
- Liquid nitrogen
- Autoclave

# Expanded Equipment

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

# Additional Supplies

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

# Introduction

- Biosafety cabinets (BSCs) are primary means of containment, developed for working safely with infectious micro-organisms

- BSCs are only one overall part of biosafety program, which requires consistent use of
  - good microbiological practices
  - primary containment equipment
  - primary containment facility design

To be precise,



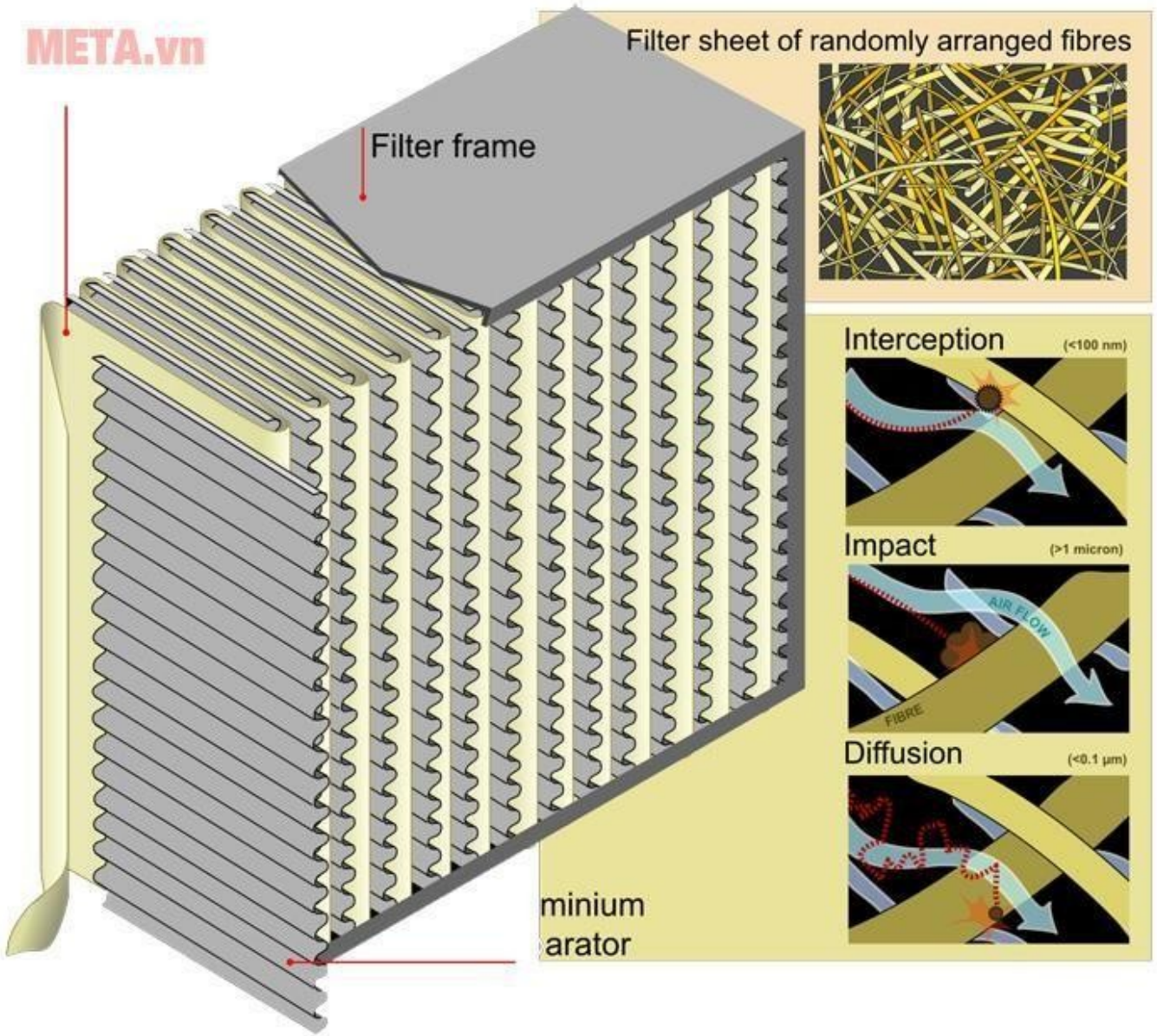

"BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed"

Adapted from CDC-BMBL- 5th Edition/1999
Appendix A – Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets

# HEPA FILTER

- HEPA – High efficiency particulate air filter
- It removes the most penetrating particle size (MPPS) of 0.3 µm with an efficiency of at least 99.97 %
- The typical HEPA filter is a single sheet of borosilicate fibers treated with a wet-strength water-repellant binder

# Hepa Filter


META.vn
Filter frame
Filter sheet of randomly arranged fibres
Interception
(<100 nm)
Impact
(>1 micron)
AIR FLOW
FIBRE
Diffusion
(<0.1 µm)
minium
arator

# Importance of a Biosafety cabinet

- Provide protection to the
  - personnel handling infectious material
  - environment by preventing the release of microbes
  - product (e.g. in handling cell cultures)

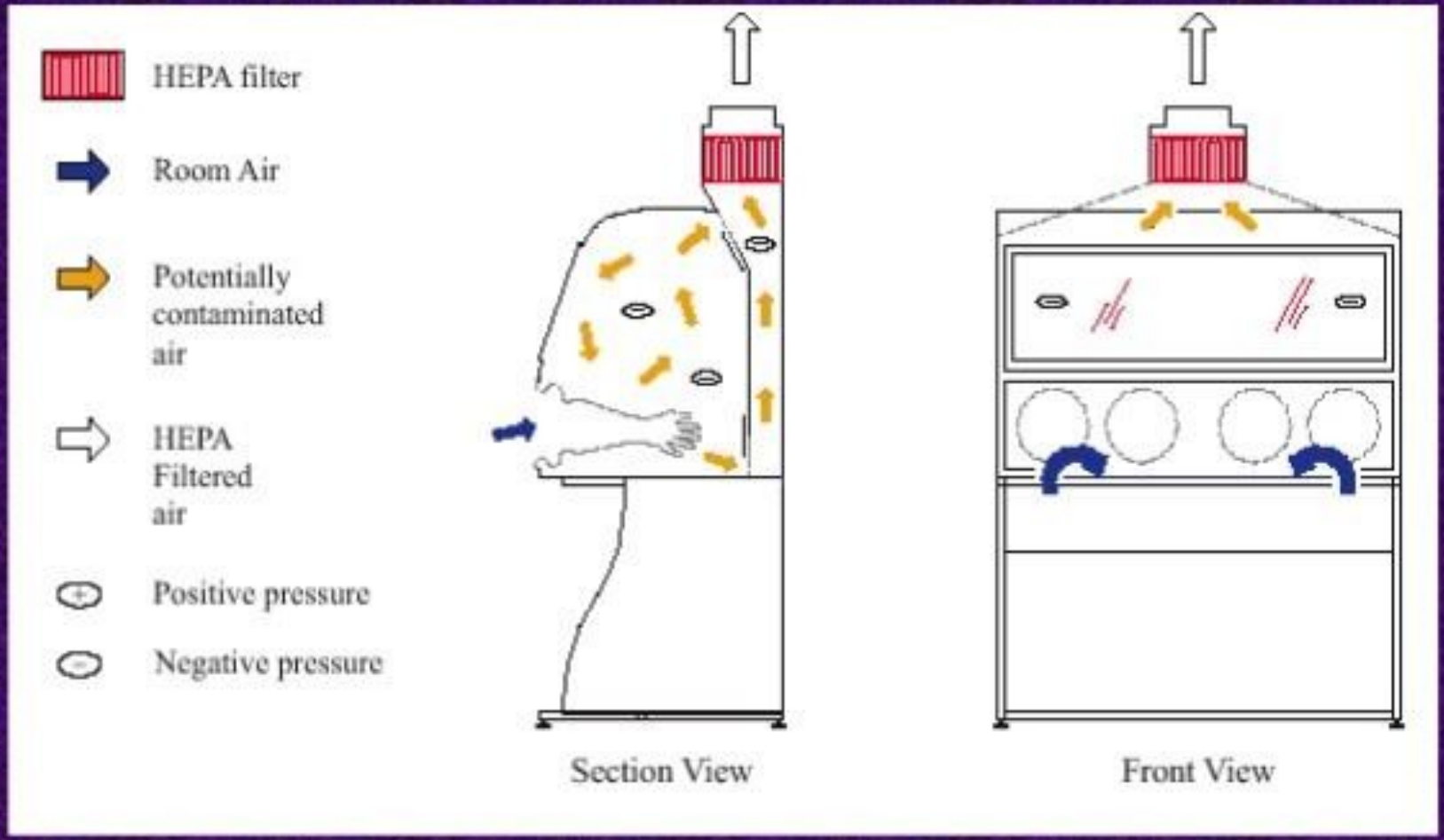
# Classes of Cell Culture Hoods (Biosafety Cabinets)

- Class I
- Class II
  - Class II A and Class II B
- Class III

# Class 1 Cabinets

- Class I cell culture hoods offer significant levels of protection to **laboratory personnel** and to **the environment** when used with good microbiological techniques,
- **But they do not provide cultures protection from contamination.**
- They are similar in design and air flow characteristics to chemical fume hoods.

# CLASS I cabinet



# Class II Cabinets

- Class II cell culture hoods are designed **for work involving BSL-1, 2, and 3 materials**, and they also provide an aseptic environment necessary for cell culture experiments.
- A Class II biosafety cabinet should be used for handling potentially hazardous materials **(e.g., primate-derived cultures, virally infected cultures, carcinogenic or toxic reagents)**.

# CLASS II A2

HEPA filter

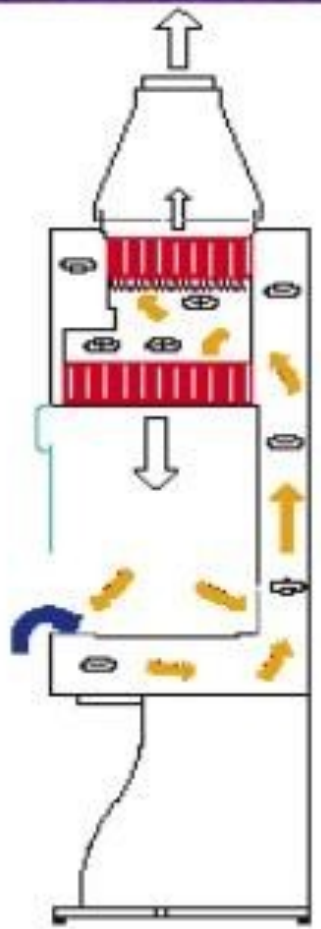
Room Air

Potentially contaminated air

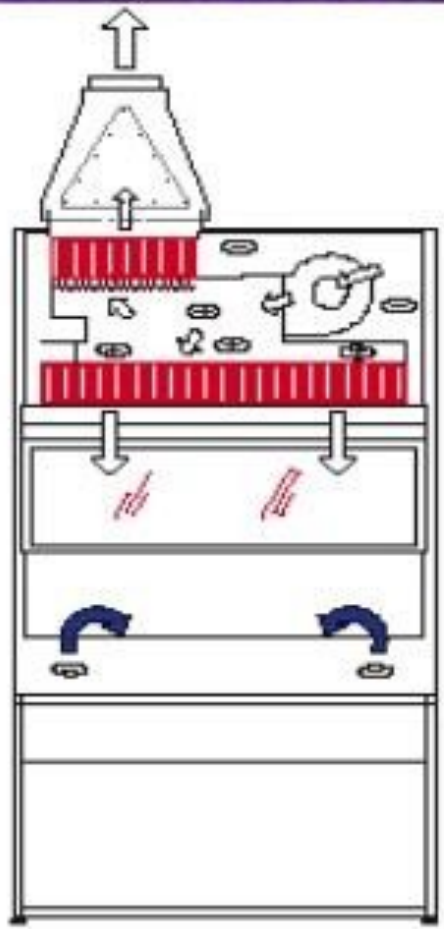
HEPA Filtered air

Positive pressure

Negative pressure



Section View



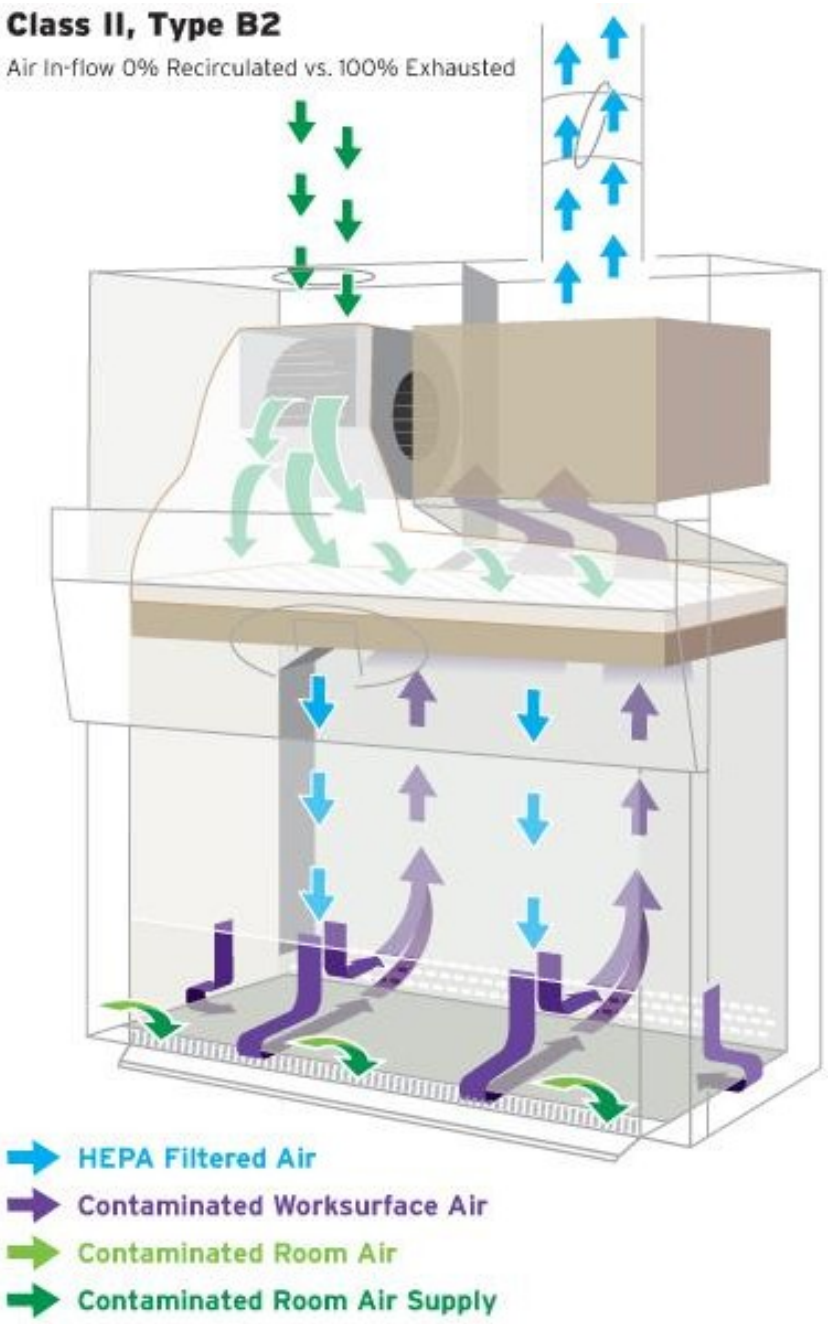
Front View

# Type II-A

- A front access opening with a carefully maintained inward flow
- HEPA-filtered unidirectional airflow
- HEPA-filtered exhaust air to the lab (30%)
- 70% of the air re-circulated back into the laminar flow hood
- Are not suitable for wok with radionuclides or volatile materials

# Type II B

- Are conducted to exterior of building
- Air are not re-circulated within the cabinets
- Suitable for work with radionuclides and volatile materials



# Class III biosafety cabinets

- Class III biosafety cabinets are gas-tight, and **they provide the highest attainable level of protection to personnel and the environment**.
- A Class III biosafety cabinet is required for work involving known **human pathogens and other BSL-4 materials.**

# Class III

HEPA filter

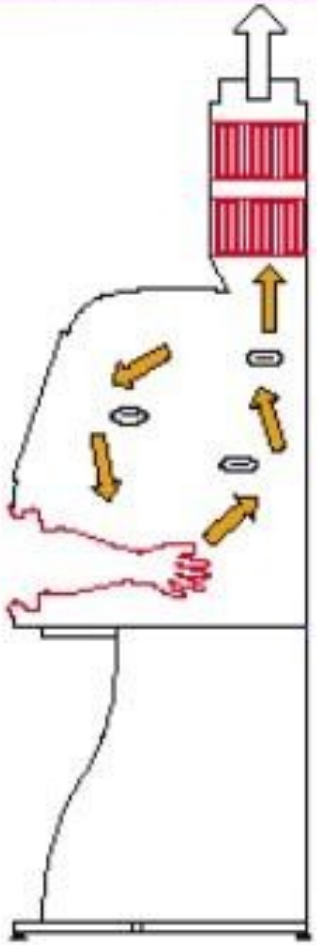
Room Air

Potentially contaminated air

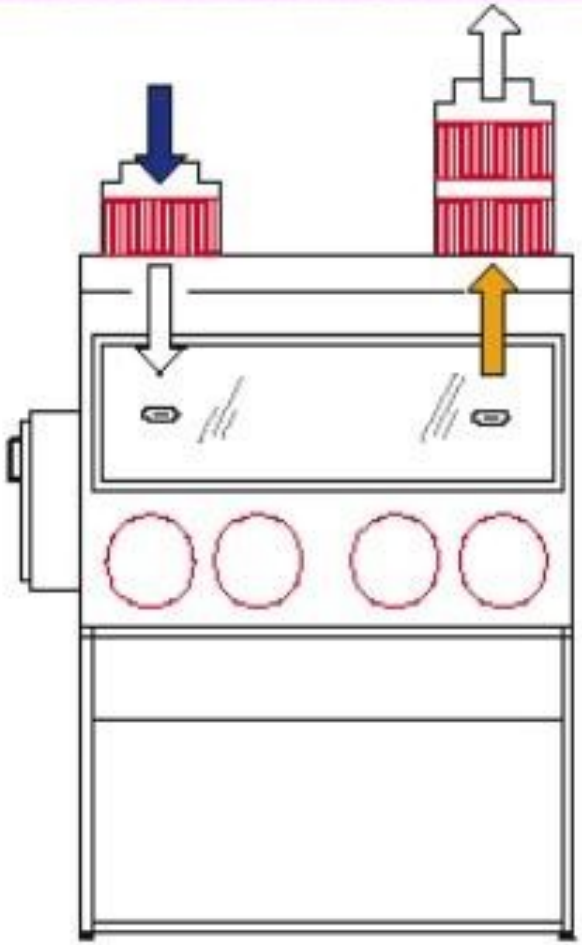
HEPA Filtered air

Positive pressure

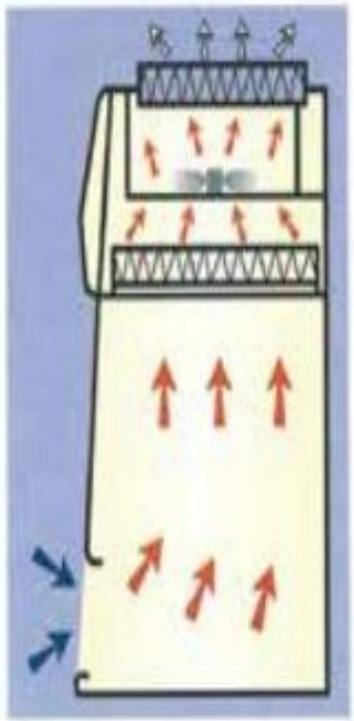
Negative pressure



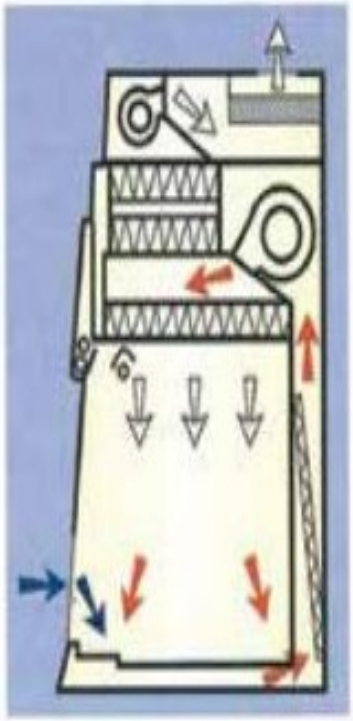
Section View



Front View



Class I



Class 2

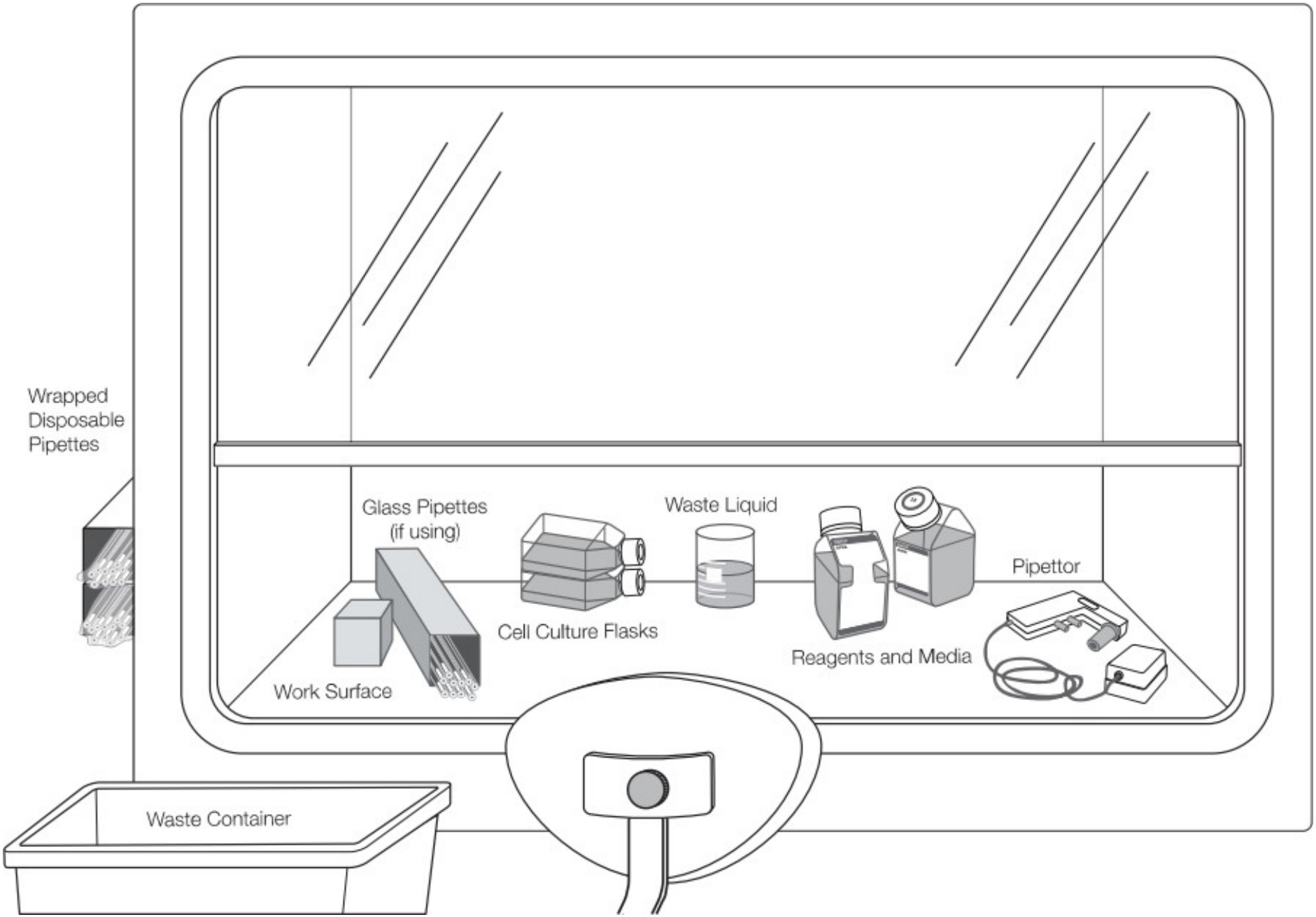
Class 3

- Environmental monitoring with Tryptose Soya Broth agar settle plates inside the cabinet for a minimum of four hours is a good indicator of how clean a cabinet is.
- **There should be no growth of bacteria , after incubation for 3 days, or fungi after incubation for 5 days, on such plates.**


- In most cases a class 2 cabinet is adequate for animal cell culture.
- However, each study must be assessed for its hazard risk and it is possible that additional factors, such as a known virus infection or an uncertain provenance may require a higher level of containment.

# Biosafety Cabinet (Area Classification)

| Class I | Class II | Class III |
| --- | --- | --- |
| • Offer signification levels of protection to laboratory personnel and to the environment when used with good microbiological technique.<br>• But they do not provide cultures protection from contamination. They are similar in design and air flow characteristics to chemical fume hoods.<br>• Used in general microbiological research with low and moderate risk agents. | • Designed for work involving BSL-1,2, and 3 materials, and they also provide an aseptic environment necessary for cell culture experiments.<br>• Used for handling potentially hazardous materials (e.g. primate-derived cultures, virally infected cultures, radioisotopes, carcinogenic or toxic reagents. | • Gas-tight, and they provide the highest attainable level of protection to personnel and the environment.<br>• A class III biosafety cabinet is required for work involving known human pathogens and other BSL- 4materials. |


Wrapped
Disposable
Pipettes
Glass Pipettes
(if using)
Waste Liquid
Pipettor
Cell Culture Flasks
Reagents and Media
Work Surface
Waste Container

# Storage

**A cell culture laboratory should have storage areas for ;**

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

- Glassware, plastics, and specialized equipment can be stored at ambient temperature on shelves and in drawers;
- However, it is important to store all media, reagents, and chemicals according to the instructions on the label.

# Refrigerators

- For small cell culture laboratories, a domestic refrigerator (preferably one without an autodefrost freezer) is an adequate and inexpensive piece of equipment **for storing reagents and media at 2–8°C.**

- For larger laboratories, **a cold room** restricted to cell culture is more appropriate.

- Make sure that the refrigerator or the cold room is **cleaned regularly to avoid contamination**

# Freezers

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

# Fridges and freezer (-20 °C)

- Both items are very important for storage of liquid media at 4 °C and for enzymes (e.g. trypsin) and some media components (e.g. glutamine and serum) at -20 °C.
- A fridge or cold room is required to store medium and buffers. A freezer will be needed for keeping pre-aliquoted stocks of serum, nutrients and antibiotics. Reagents may be stored at -20 °C.
- For prolonged preservation, cells may be preserved in liquid nitrogen or a -70 °C freezer.

# Fridges and freezer (-20 °C)

# Incubators

- Cell cultures require a strictly controlled environment in which to grow.
- Specialist incubators are used routinely to provide the correct growth conditions, such as temperature, degree of humidity and CO2 levels in a controlled and stable manner.

- Generally, they can be set to run at temperatures in the range of 28 $^{o}$C (for insect cell lines) to **37 $^{o}$C (for mammalian cell lines)** and set to provide $CO_2$ at the required level (e.g. 5-10%).
- Some incubators also have the facility to control the O2 levels.

- There are two basic types of incubators:

1. **dry incubators**
2. **humid CO2 incubators.**


- Dry incubators are more economical, but require the cell cultures to be incubated in sealed flasks to prevent evaporation.
- Placing a water dish in a dry incubator can provide some humidity, but they do not allow precise control of atmospheric conditions in the incubator.
- Humid CO2 incubators are more expensive, **but allow superior control of culture conditions.**
- They can be used to incubate cells cultured in Petri dishes or multiwell plates, which require a controlled atmosphere of high humidity and increased CO2 tension.

# 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 decreases when they are stored at these temperatures.**

# CRITICAL EQUIPMENT (Cryogenic Storage)

Cryogenic Storage

- Two main types of liquid-nitrogen storage systems, **vapor phase** and **liquid phase**.
- **Vapor phase-** minimize the risk of explosion with cryostorage tubes, and are required for storing biohazardous materials.
- **Liquid phase-** usually have longer static holding times, more economical.
- **Narrow-necked containers-** slower nitrogen evaporation rate, economical.
- **Wide-necked containers-** allow easier access and have a larger storage capacity.

Vapor phase


Liquid Phase

# Cooling centrifuge

- Centrifuge is an instrument used to separate the particles from a solution according to their size, shape, density, viscosity of the medium and the rotor speed by using the principle of centrifugal force.
- Cooling centrifuge is used to prepare the cell for cryo-preservation.

# Inverted microscope

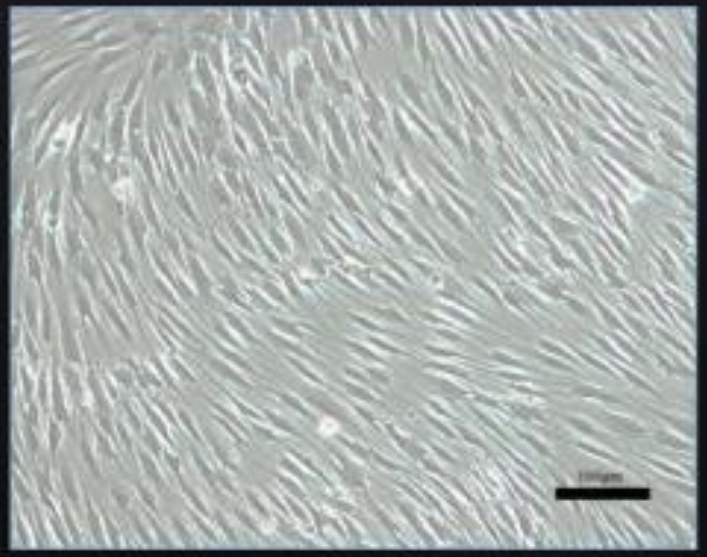
- An **inverted microscope** is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up.
- Inverted microscopes are useful for observing living cells at the bottom of a large container (e.g., a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope.

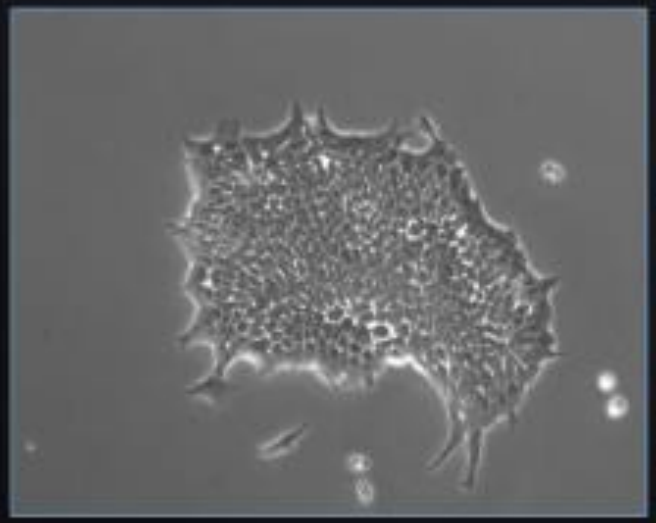
# Inverted Microscope

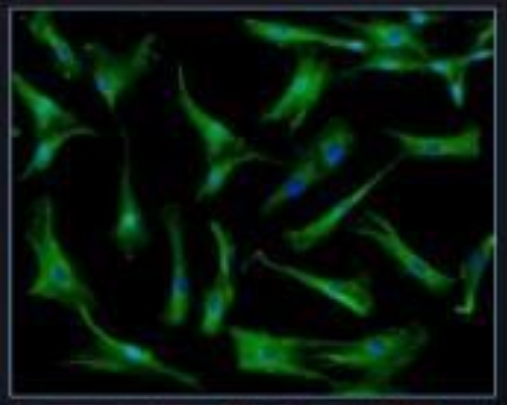
Inverted microscope

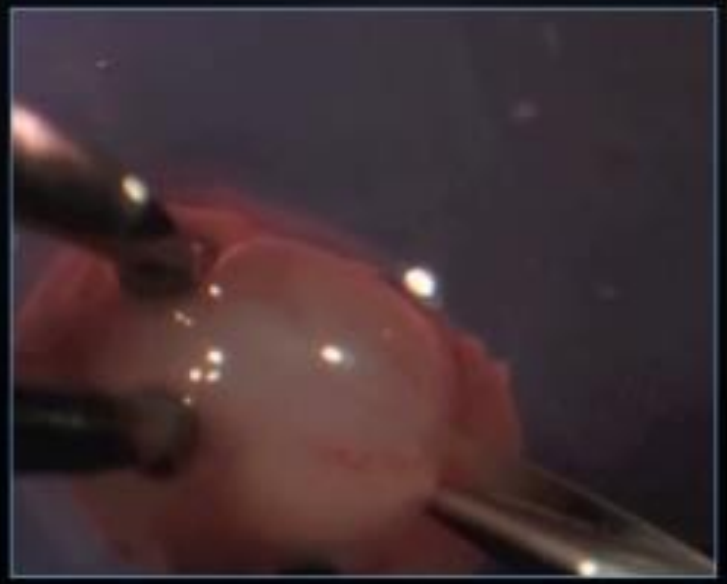
Fluorescent inverted microscope

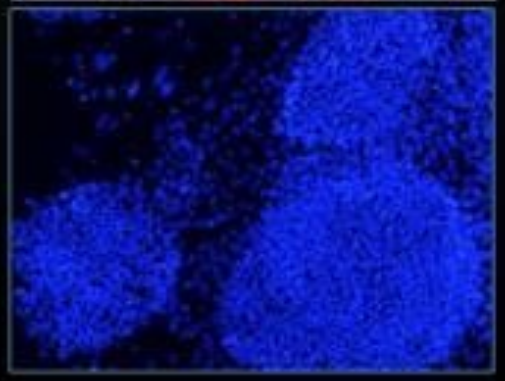
Stereo or dissecting microscope









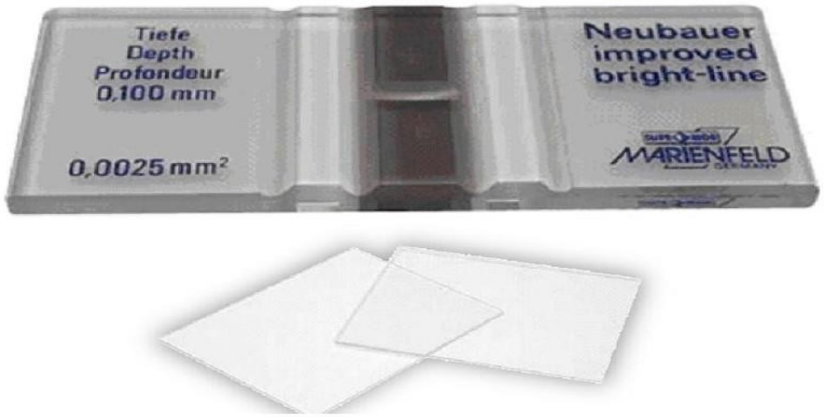


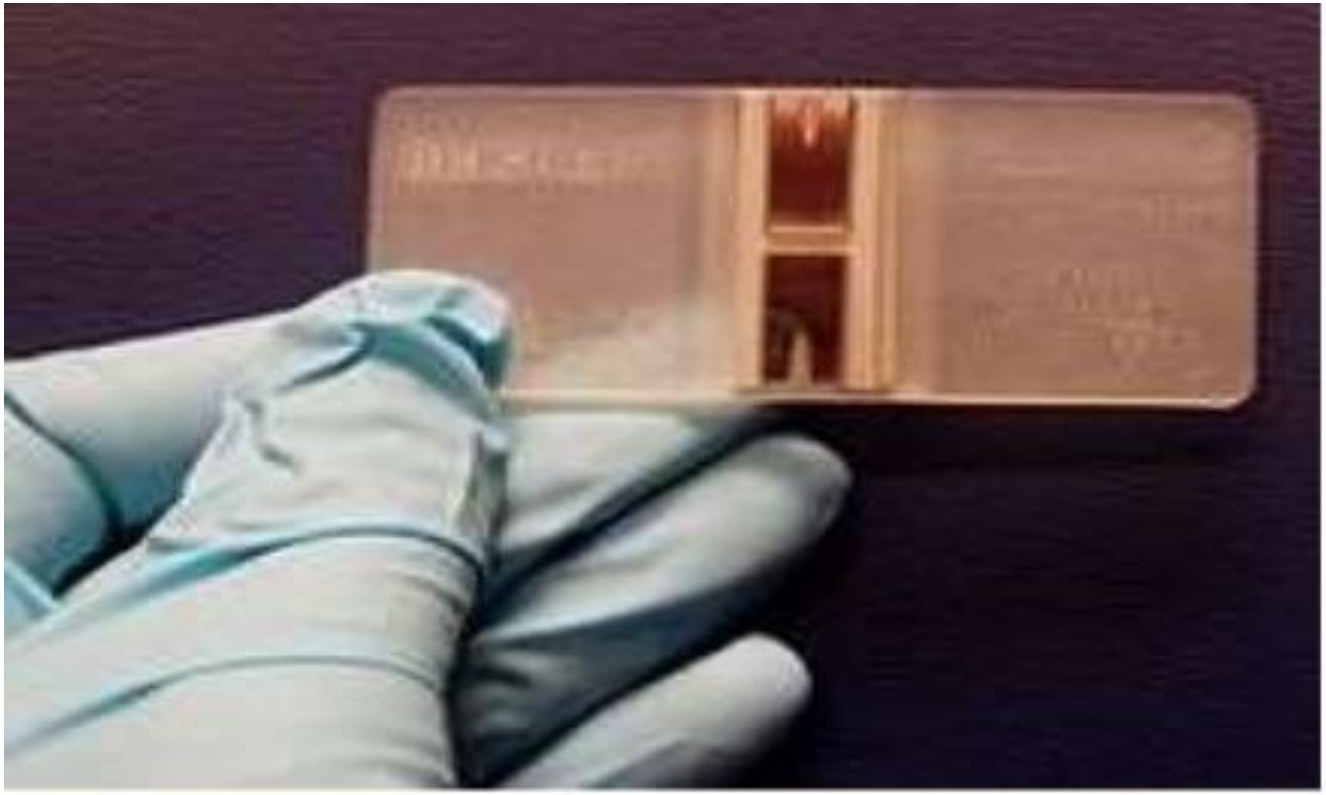
# Sterilizer (autoclave)


An **autoclave** is a pressure chamber used to carry out sterilization processes under elevated temperature and pressure .

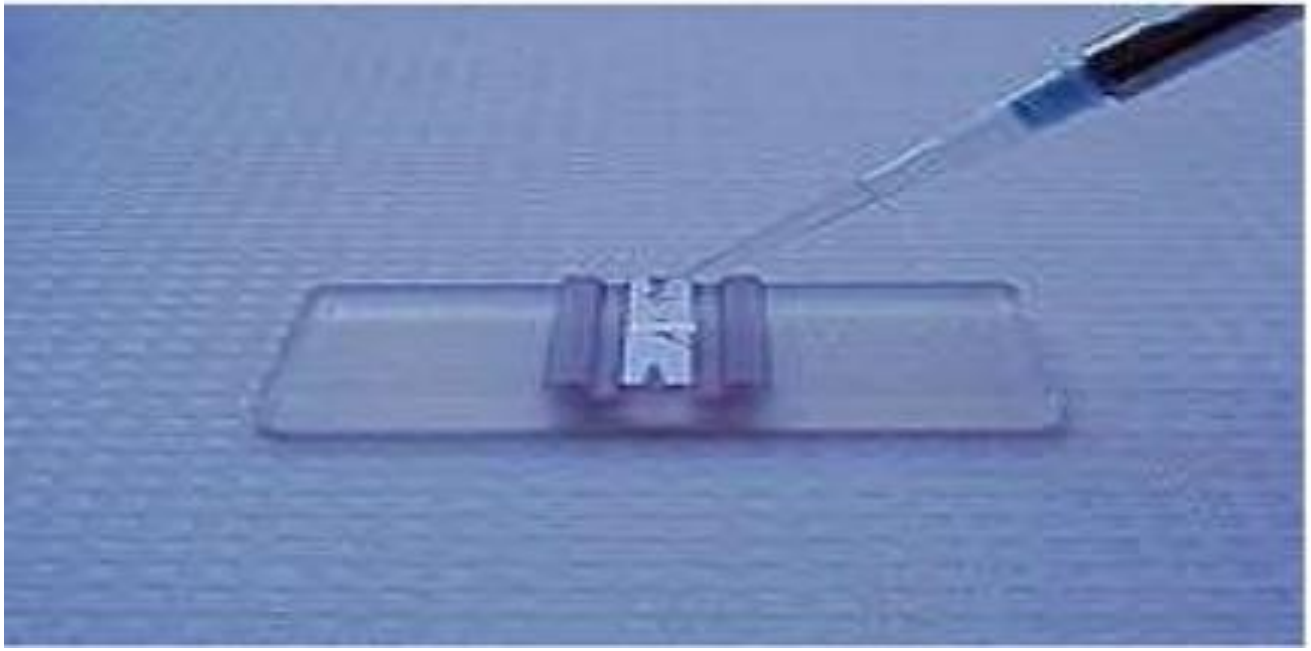
# Cell Counter (Haemocytometer)

- A cell counter is essential for quantitative growth kinetics, and a great advantage when more than two or three cell lines are cultured in the laboratory.



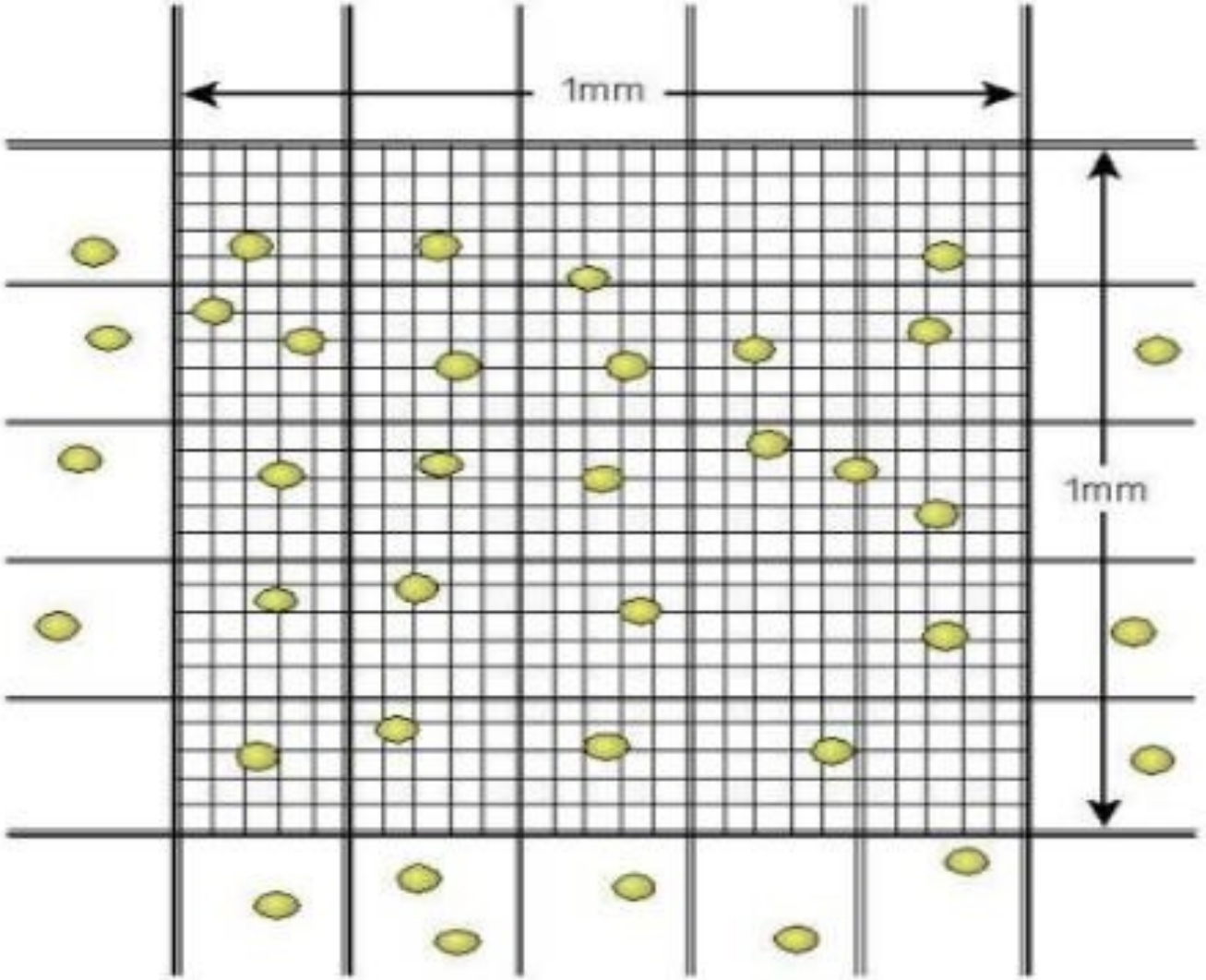


The two semi-reflective rectangles are the counting chambers.



Load a chamber

# Close up view of a grid with cells



# Polystyrene Flasks for tissue culture

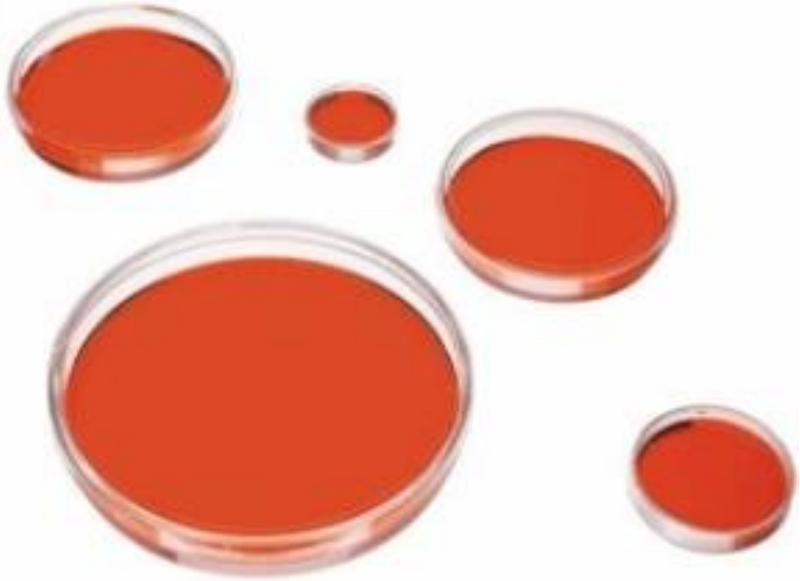
# Culture Vessels

- Flasks
  - Plastic flasks are available with a range of growing areas, a variety of shapes, with several different neck designs
  - Flasks surfaces are specially treated for growing anchorage-dependent cells

| Description | Growth area ($cm^2$) | Recommended working volume (mL) | Cell yield* |
|---|---|---|---|
| T-25 | 25 | 5 – 10 | $2.5 \times 10^6$ |
| T-75 | 75 | 15 – 25 | $7.5 \times 10^6$ |
| T-150 | 150 | 30 - 50 | $15 \times 10^6$ |
| T-175 | 175 | 35 - 60 | $17.5 \times 10^6$ |
| T-225 | 225 | 45 - 75 | $22.5 \times 10^6$ |

*Cell line dependent. Based upon a density of $1 \times 10^5$ cells/$cm^2$.

# Culture Vessels



- **Cell culture dishes**
  - Cell culture dishes offer the best economy and access to the growth surface
  - Cell culture dishes surfaces are specially treated for growing anchorage-dependent cells

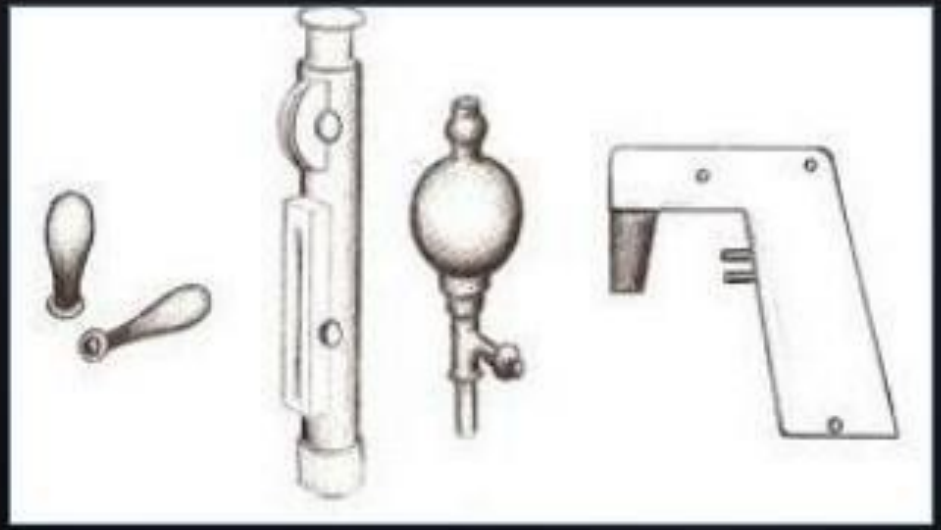
| Description | Growth area ($cm^2$) | Recommended working volume (mL) | Cell yield* |
|---|---|---|---|
| 35 | 8 | 1 - 2 | $0.8 \times 10^6$ |
| 60 | 21 | 4 - 5 | $2.1 \times 10^6$ |
| 100 | 55 | 10 - 12 | $5.5 \times 10^6$ |
| 150 | 148 | 28 - 32 | $14.8 \times 10^6$ |

*Cell line dependent. Based upon a density of $1 \times 10^5$ cells/$cm^2$.

# Culture Vessels

- **Multiwell plates**
  - Multiwell plates offer significant savings in space, media, and reagents when compared to an equal number of dishes

# Pipette aid

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