

# Cell Culture Systems

## Course 2

### Cell Culture Equipments

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# 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 }

**BIOHAZARD**



Biosafety Level 2

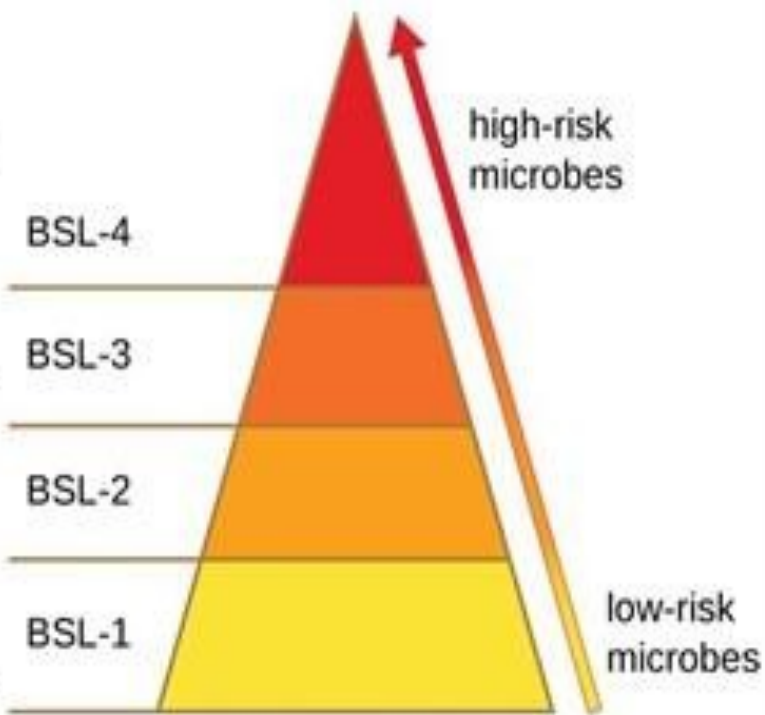
AUTHORIZED PERSONNEL ONLY

# **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	 <p>high-risk microbes</p> <p>low-risk microbes</p> <p>BSL-4</p> <p>BSL-3</p> <p>BSL-2</p> <p>BSL-1</p>
BSL-3	Microbes are indigenous or exotic and cause serious or potentially lethal diseases through respiratory transmission.	<i>Mycobacterium tuberculosis</i>	
BSL-2	Microbes are typically indigenous and are associated with diseases of varying severity. They pose moderate risk to workers and the environment.	<i>Staphylococcus aureus</i>	
BSL-1	Microbes are not known to cause disease in healthy hosts and pose minimal risk to workers and the environment.	Nonpathogenic strains of <i>Escherichia coli</i>	

# 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.

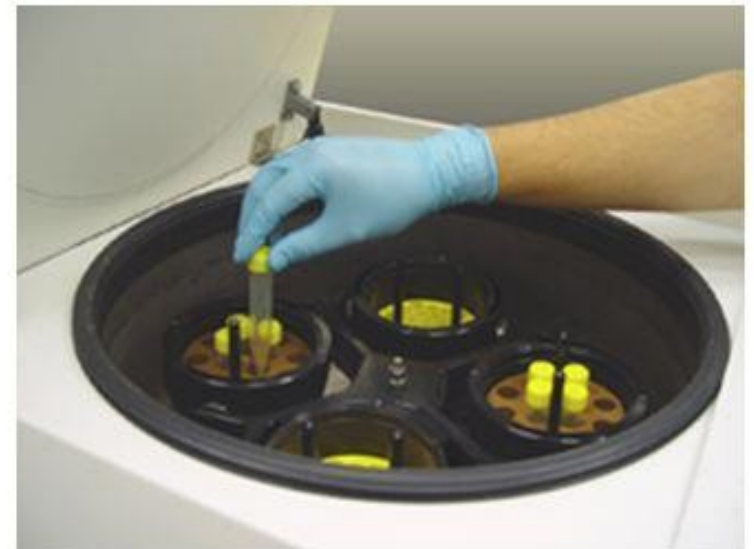


# BSL 4 - practices

- 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 ( $-20^{\circ}\text{C}$ )
- 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- 5<sup>th</sup> Edition/1999

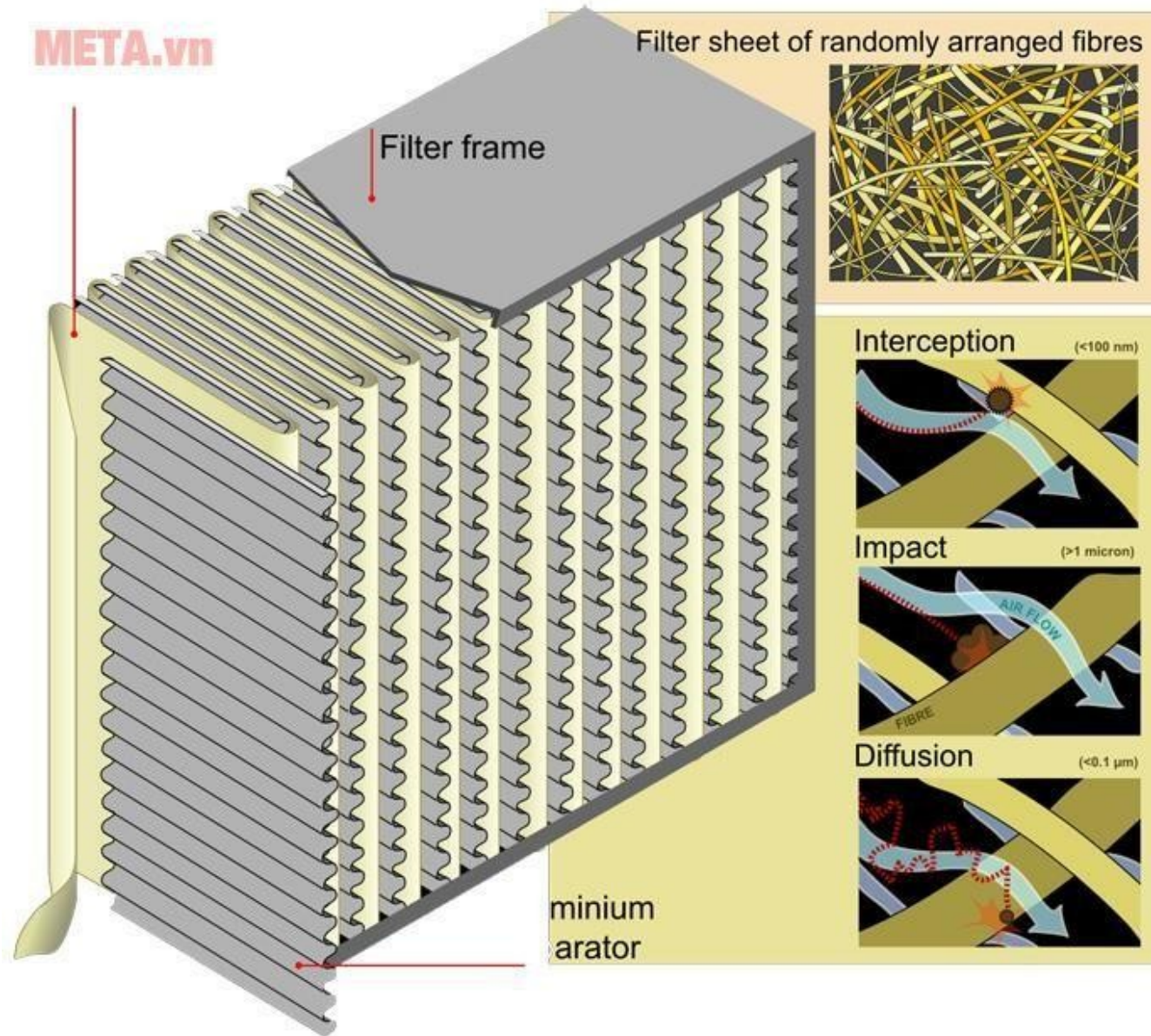
Appendix A – Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets

# HEPA FILTER

- HEPA – High efficiency particulate air filter
- It removes the most penetrating particle size (MPPS) of  $0.3\text{ }\mu\text{m}$  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



# 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)

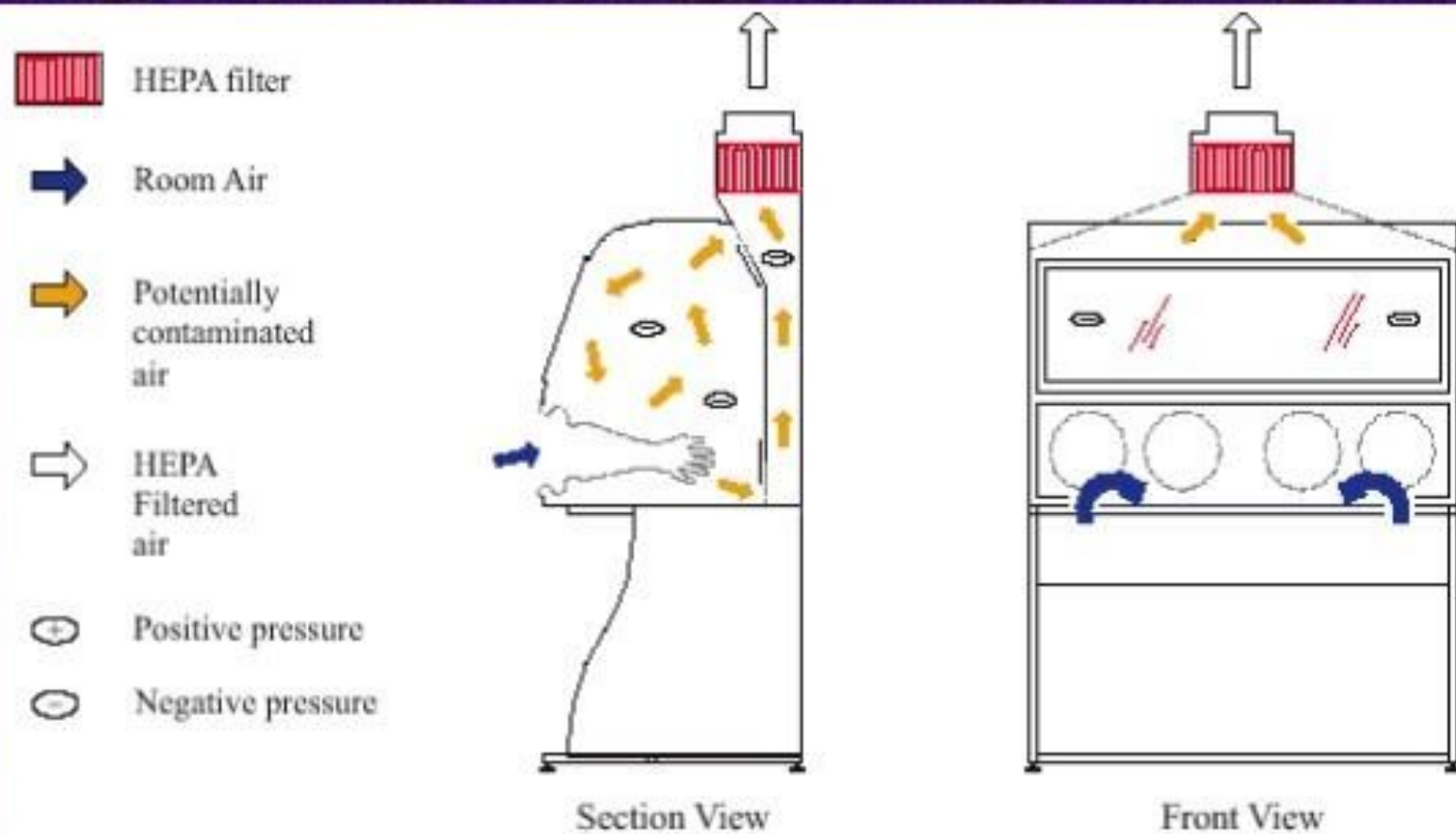
- Class I
- Class II
  - Class II A and Class II B
- Class III



# 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.

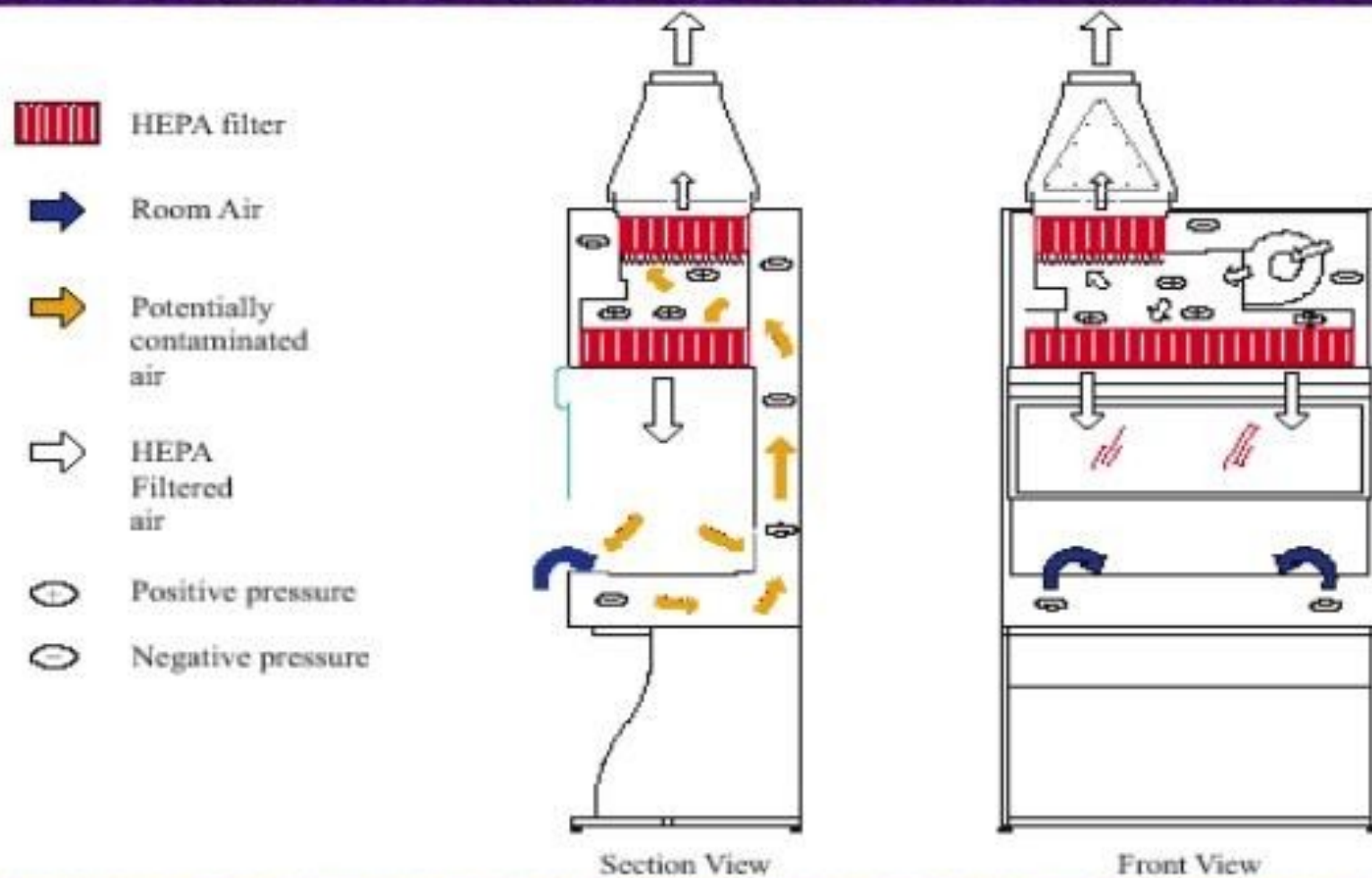
# CLASS I cabinet



# 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



## Type II-A

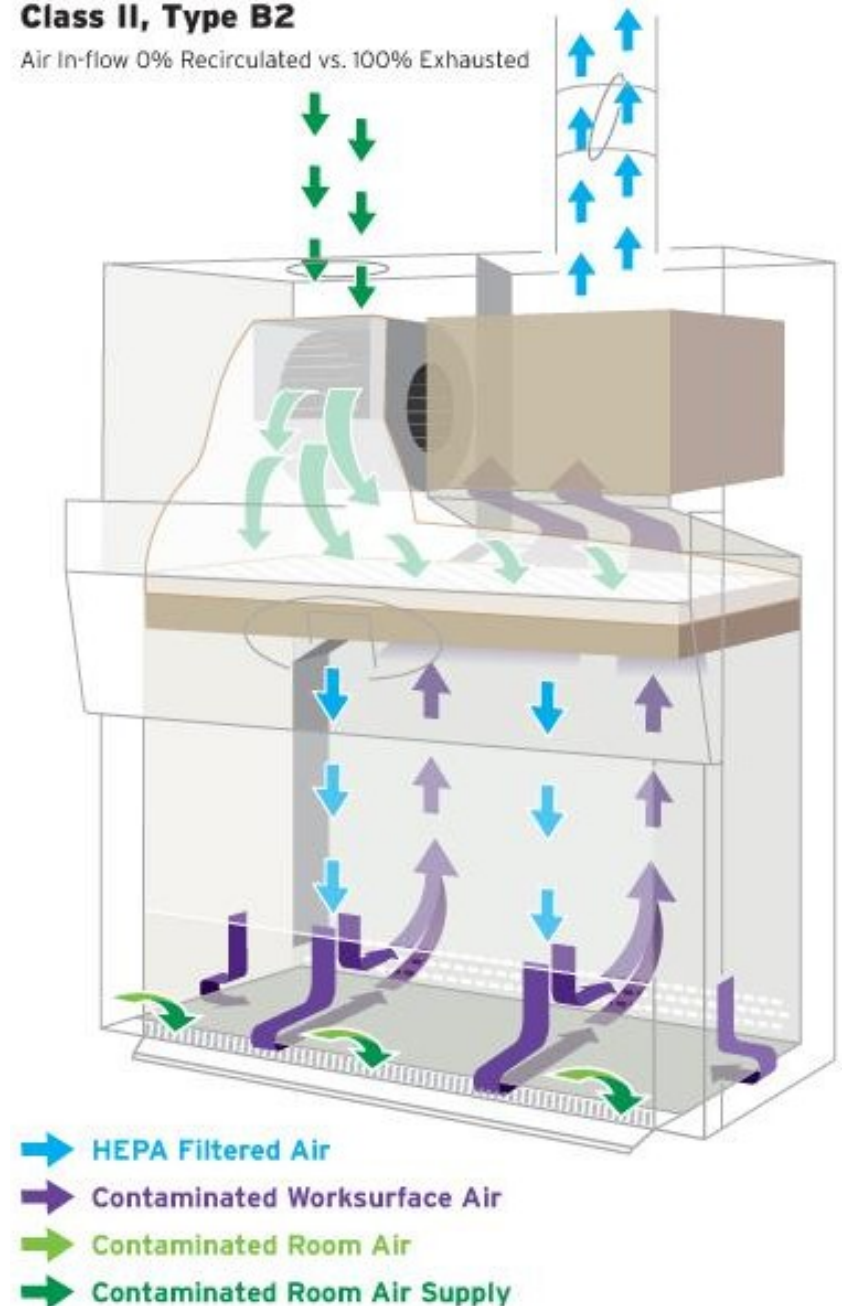
- A front access opening with a carefully maintained inward flow
- HEPA-filtered unidirectional airflow
- HEPA-filtered exhaust air to the lab (30%)
- 70% of the air re-circulated back into the laminar flow hood
- Are not suitable for work with radionuclides or volatile materials

# 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 II, Type B2

Air In-flow 0% Recirculated vs. 100% Exhausted

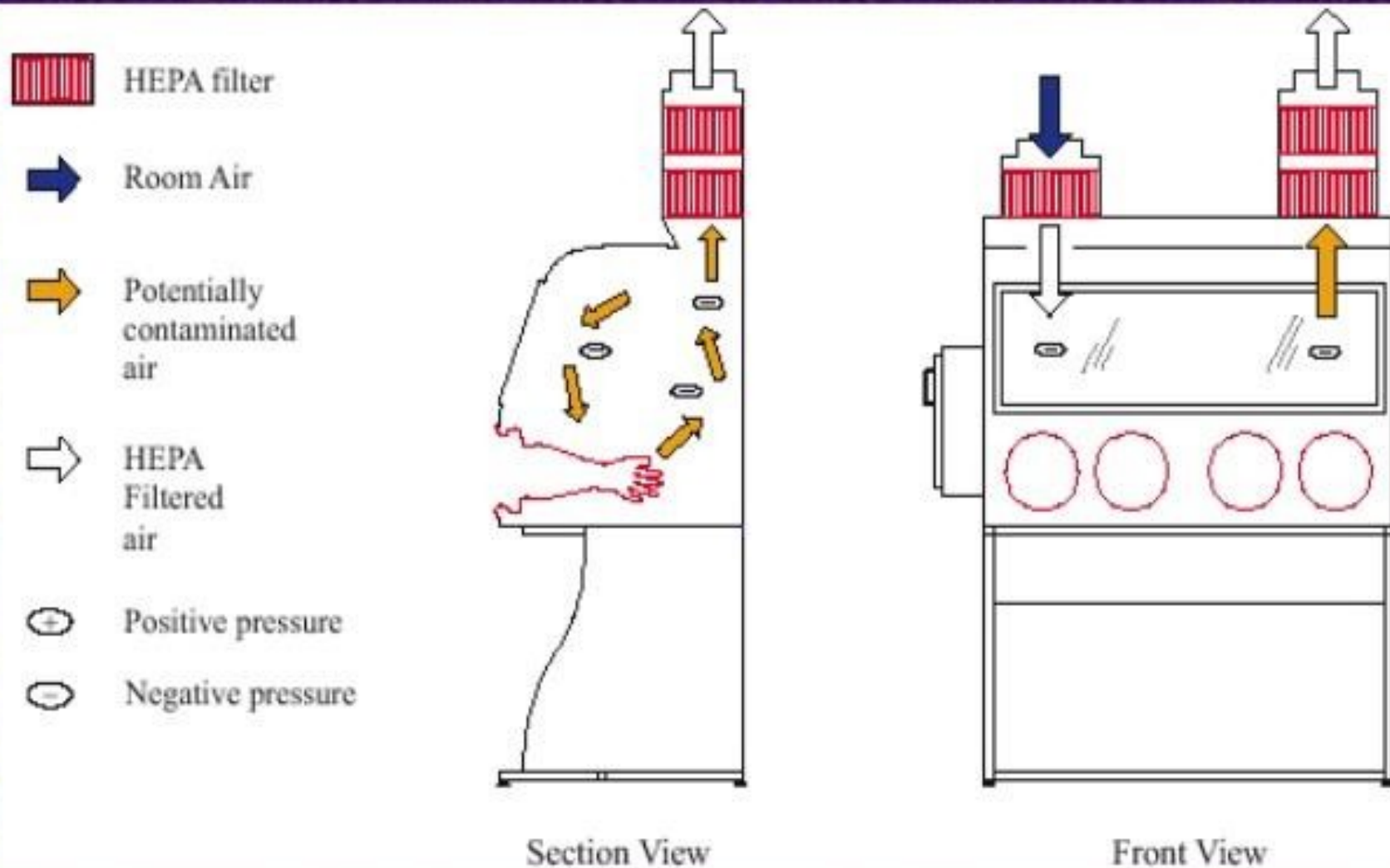


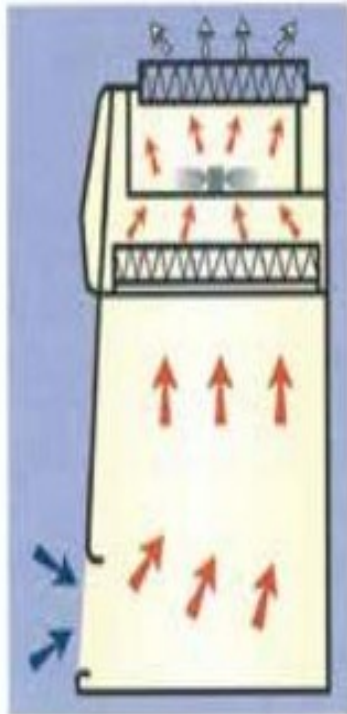


# Class III biosafety cabinets

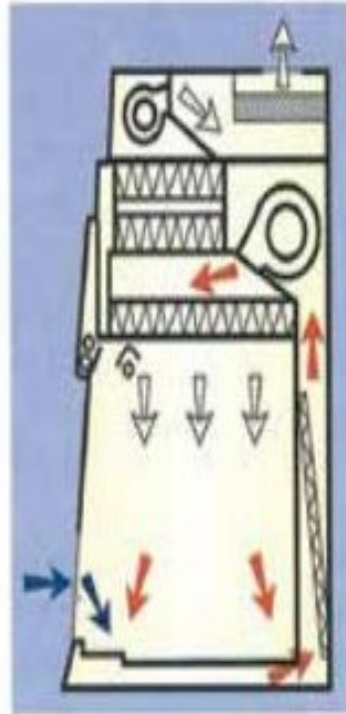
- Class III biosafety cabinets are gas-tight, and they provide the highest attainable level of protection to personnel and the environment.
- A Class III biosafety cabinet is required for work involving known human pathogens and other BSL-4 materials.

# Class III





Class I



Class 2



Class 3



# Air-flow characteristics of cell culture hoods

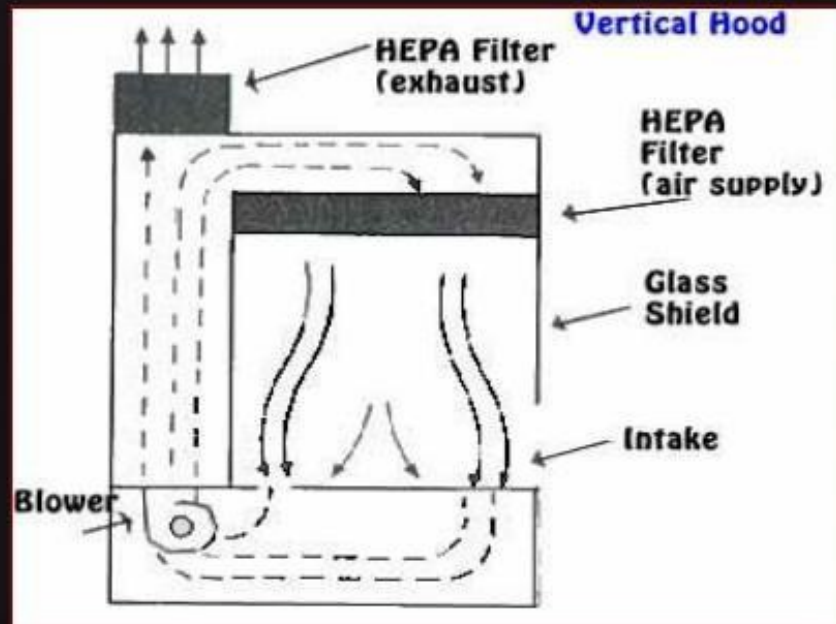
- Cell culture hoods protect the working environment from dust and other airborne contaminants by maintaining a constant, unidirectional flow of HEPA-filtered air over the work area.
- The flow can be horizontal, blowing parallel to the work surface, or it can be vertical, blowing from the top of the cabinet onto the work surface.

# Air-flow characteristics of cell culture hoods

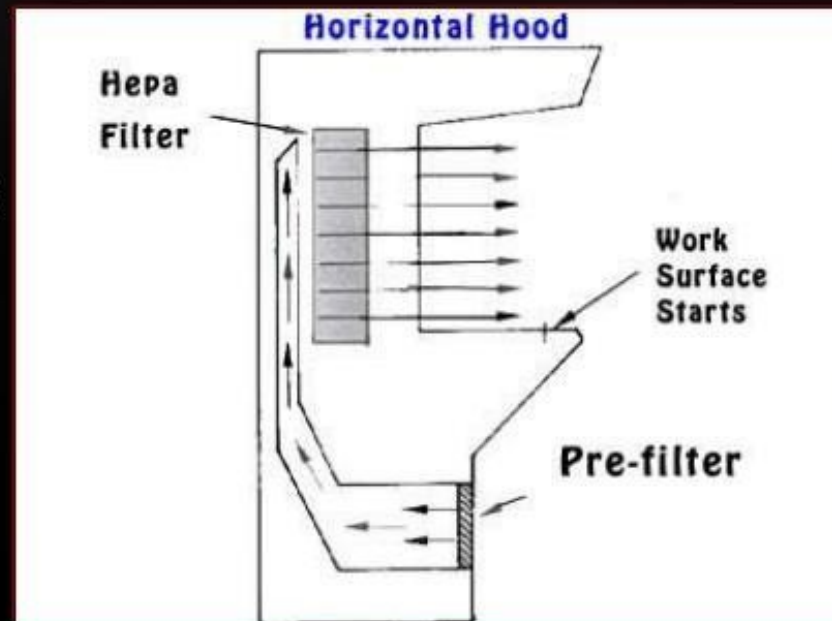
- Depending on its design, a horizontal flow hood provides protection to the culture (if the air flowing towards the user) or to the user (if the air is drawn in through the front of the cabinet by negative air pressure inside).
- Vertical flow hoods, on the other hand, provide significant protection to both the user and the cell culture.

# Laminar flow hood (Biological safety cabinet)

## 1. Vertical mode



## 2. Horizontal mode





- 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

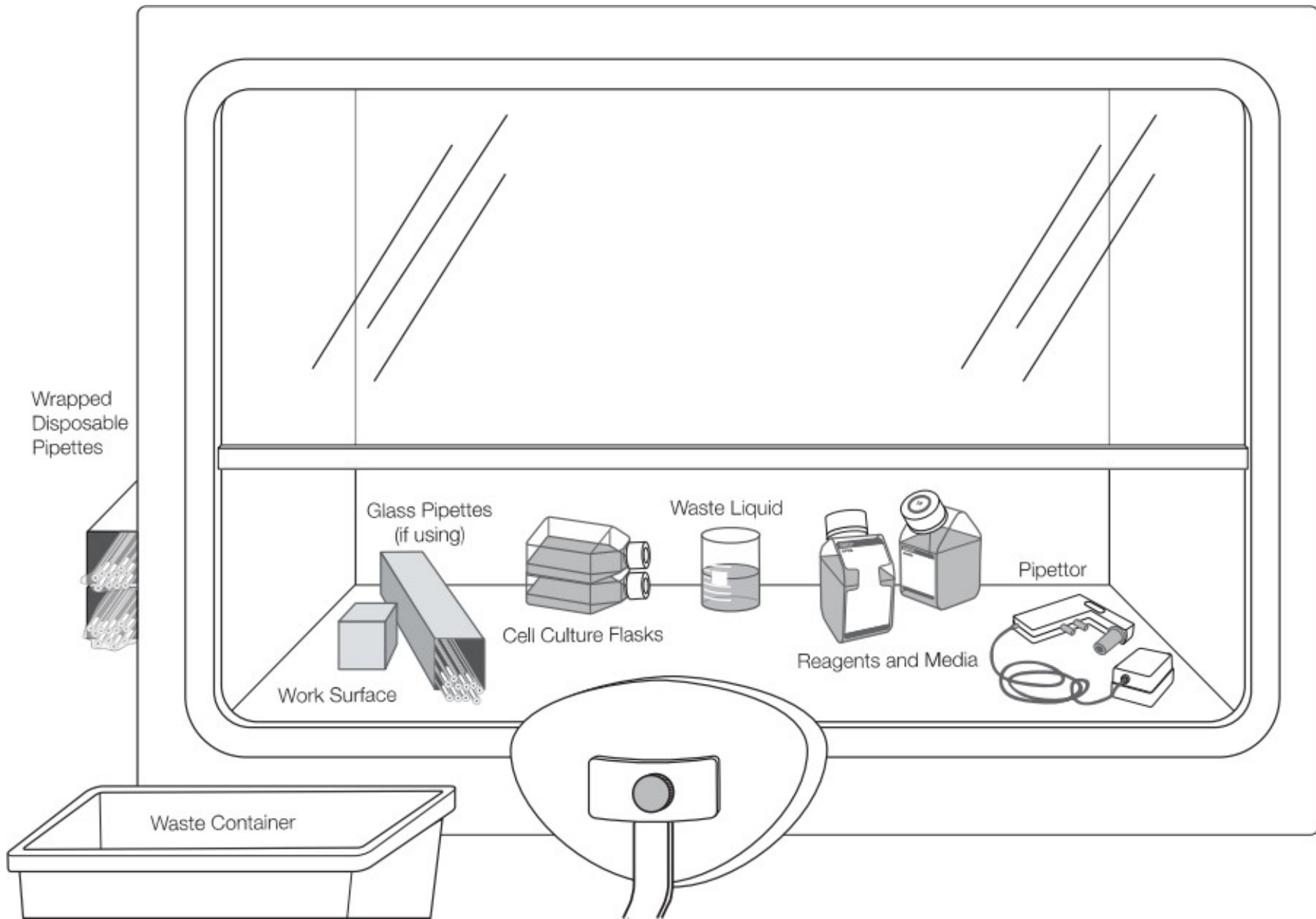
- Offer signification levels of protection to laboratory personnel and to the environment when used with good microbiological technique.
- But they do not provide cultures protection from contamination. They are similar in design and air flow characteristics to chemical fume hoods.
- Used in general microbiological research with low and moderate risk agents.

## Class II

- Designed for work involving BSL-1,2, and 3 materials, and they also provide an aseptic environment necessary for cell culture experiments.
- Used for handling potentially hazardous materials (e.g. primate-derived cultures, virally infected cultures, radioisotopes, carcinogenic or toxic reagents).

## Class III

- 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- 4materials.



# 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  $-5^{\circ}\text{C}$  to  $-20^{\circ}\text{C}$ ; therefore, an ultradeep freezer (i.e., a  $-80^{\circ}\text{C}$  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 CO<sub>2</sub> levels in a controlled and stable manner.



- Generally, they can be set to run at temperatures in the range of 28 °C (for insect cell lines) to 37 °C (for mammalian cell lines) and set to provide CO<sub>2</sub> at the required level (e.g. 5-10%).
- Some incubators also have the facility to control the O<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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



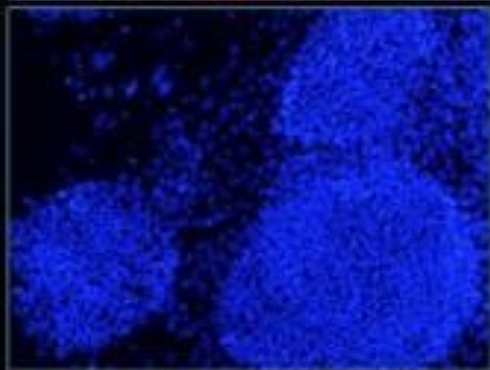
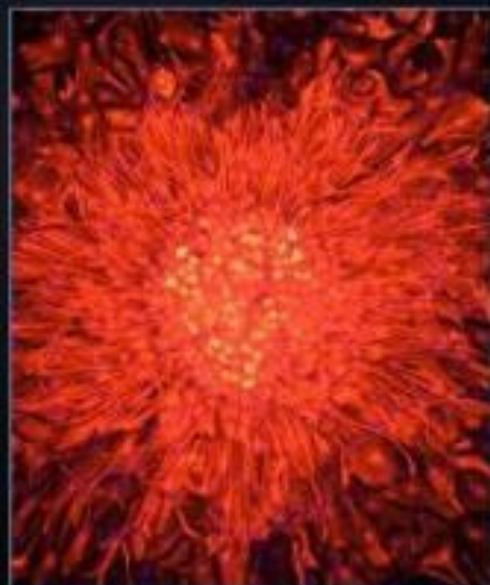
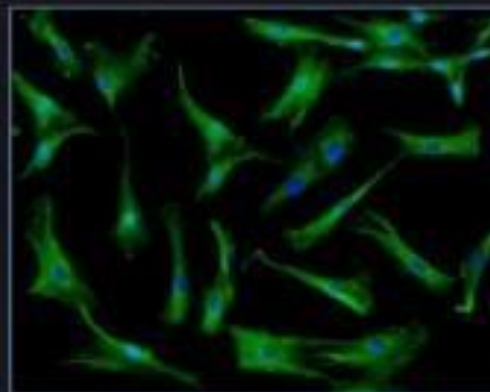
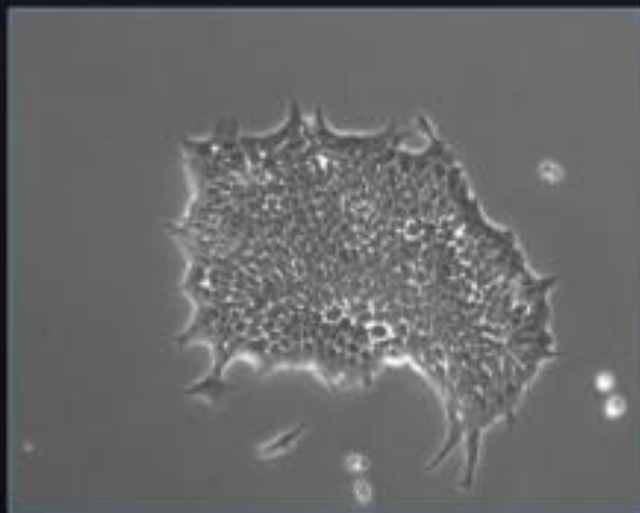
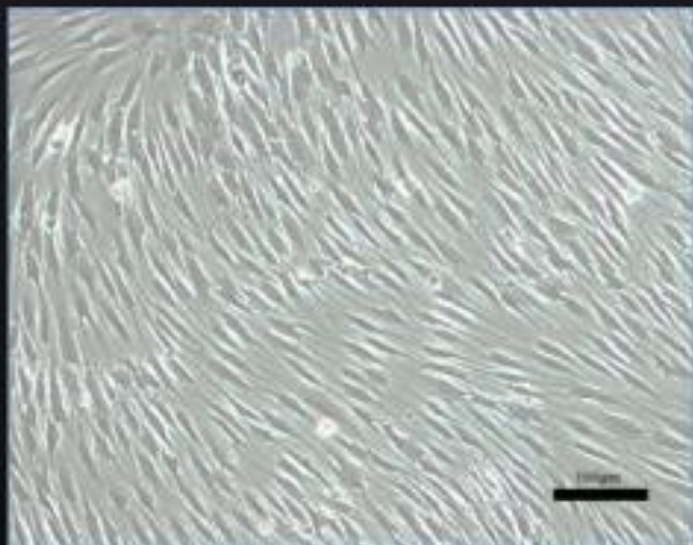
Inverted microscope



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 .



# Expanded Equipment

- • Aspiration pump
- • pH meter



• Aspiration pump



pH meter

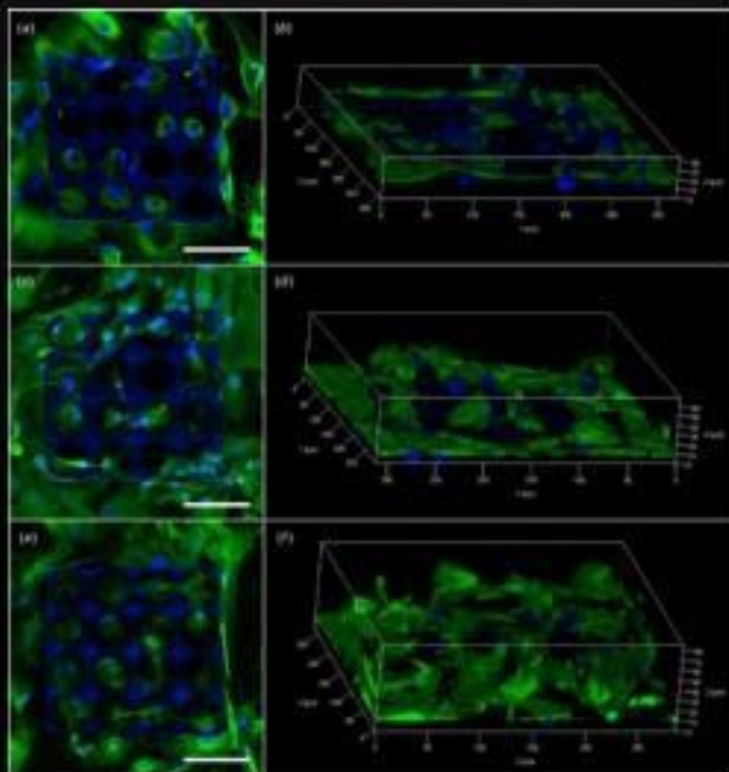
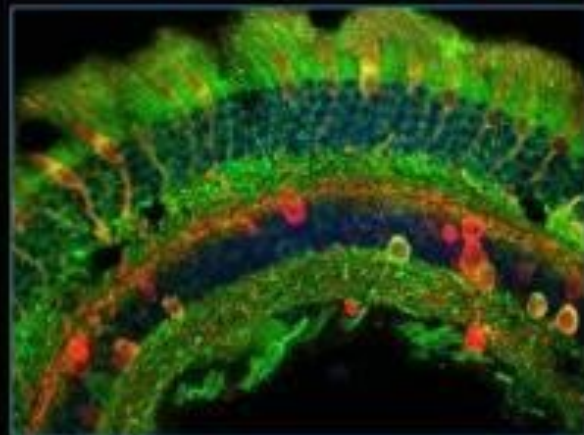
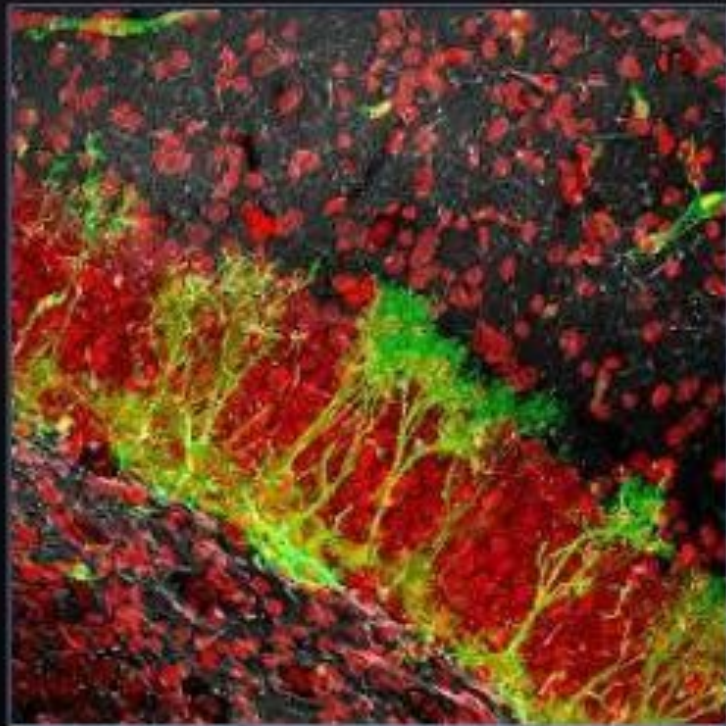
## ■ Confocal microscope

**Confocal microscopy** is an optical imaging technique for increasing optical resolution and contrast of a micrograph. It enables the reconstruction of three-dimensional structures from the obtained images.





# Confocal microscopy



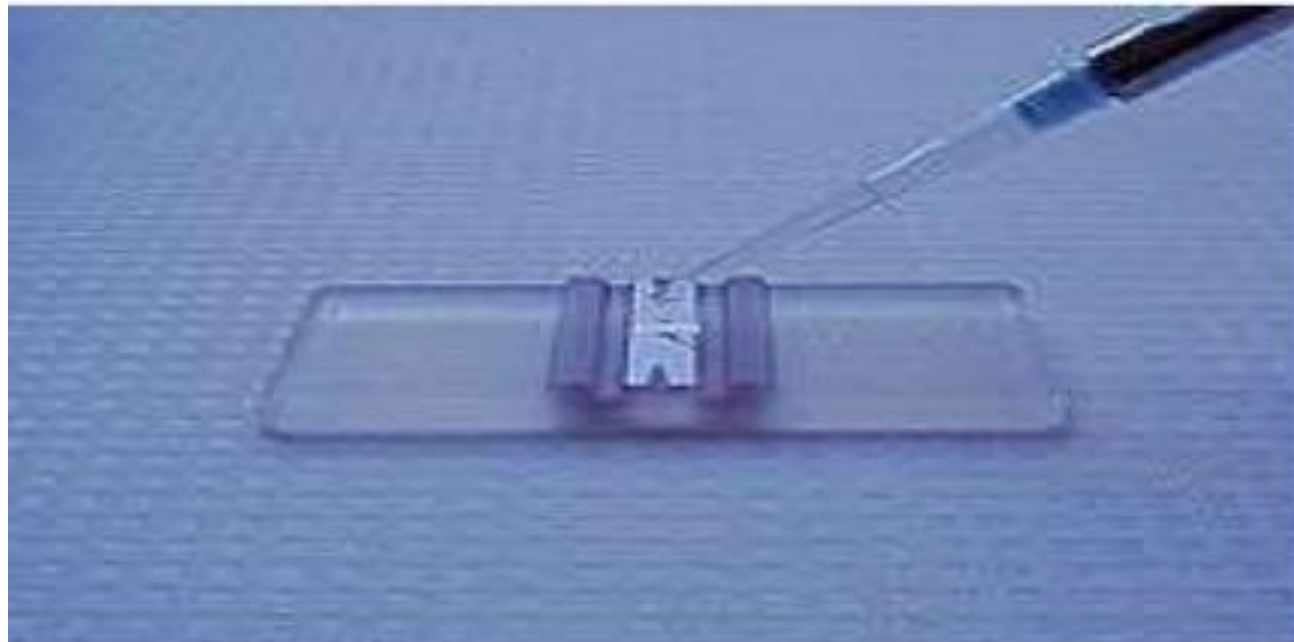
# Cell Counter (Haemocytometer)

- 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.



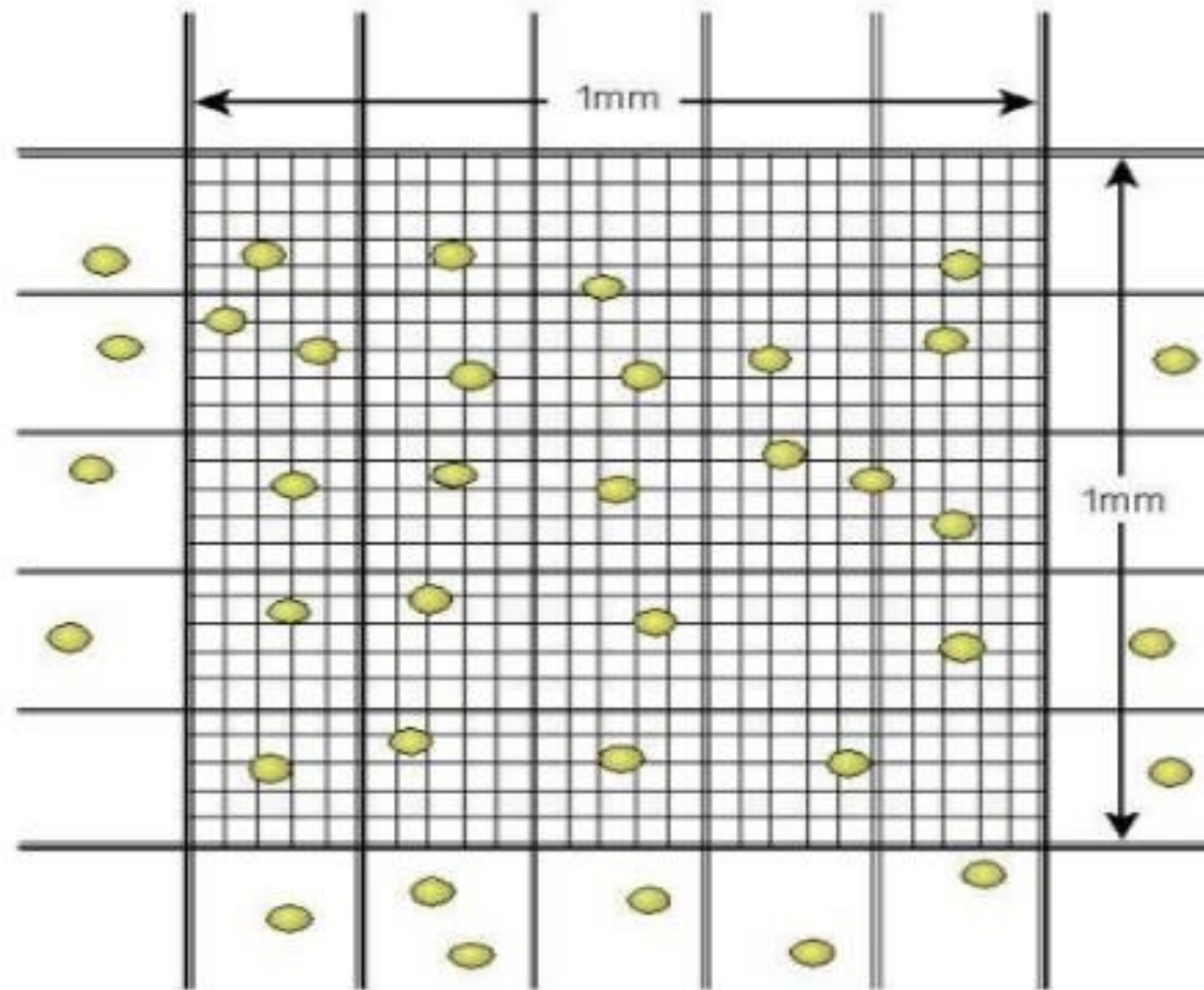


The two semi-reflective rectangles are the counting chambers.



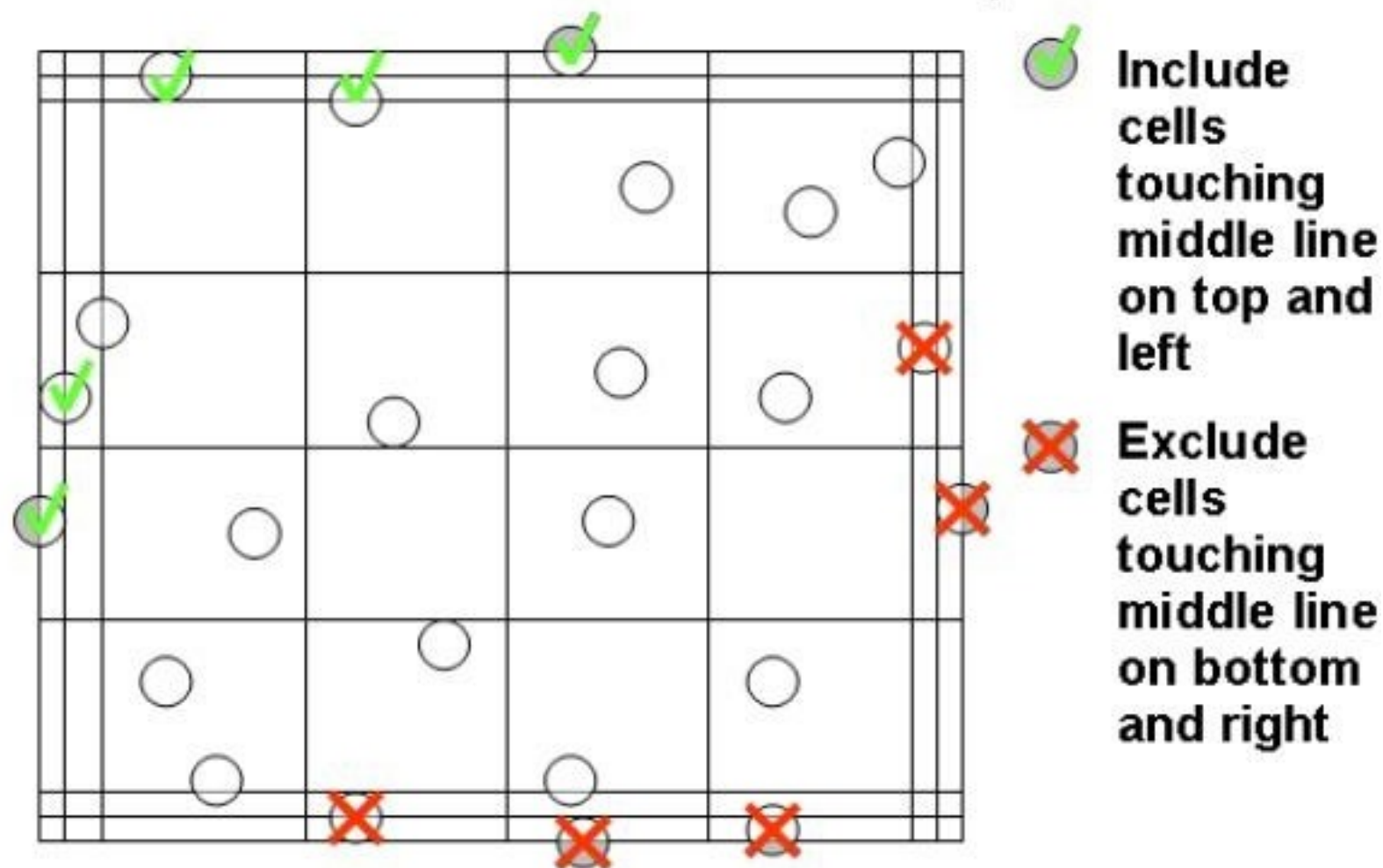
Load a chamber

## Close up view of a grid with cells



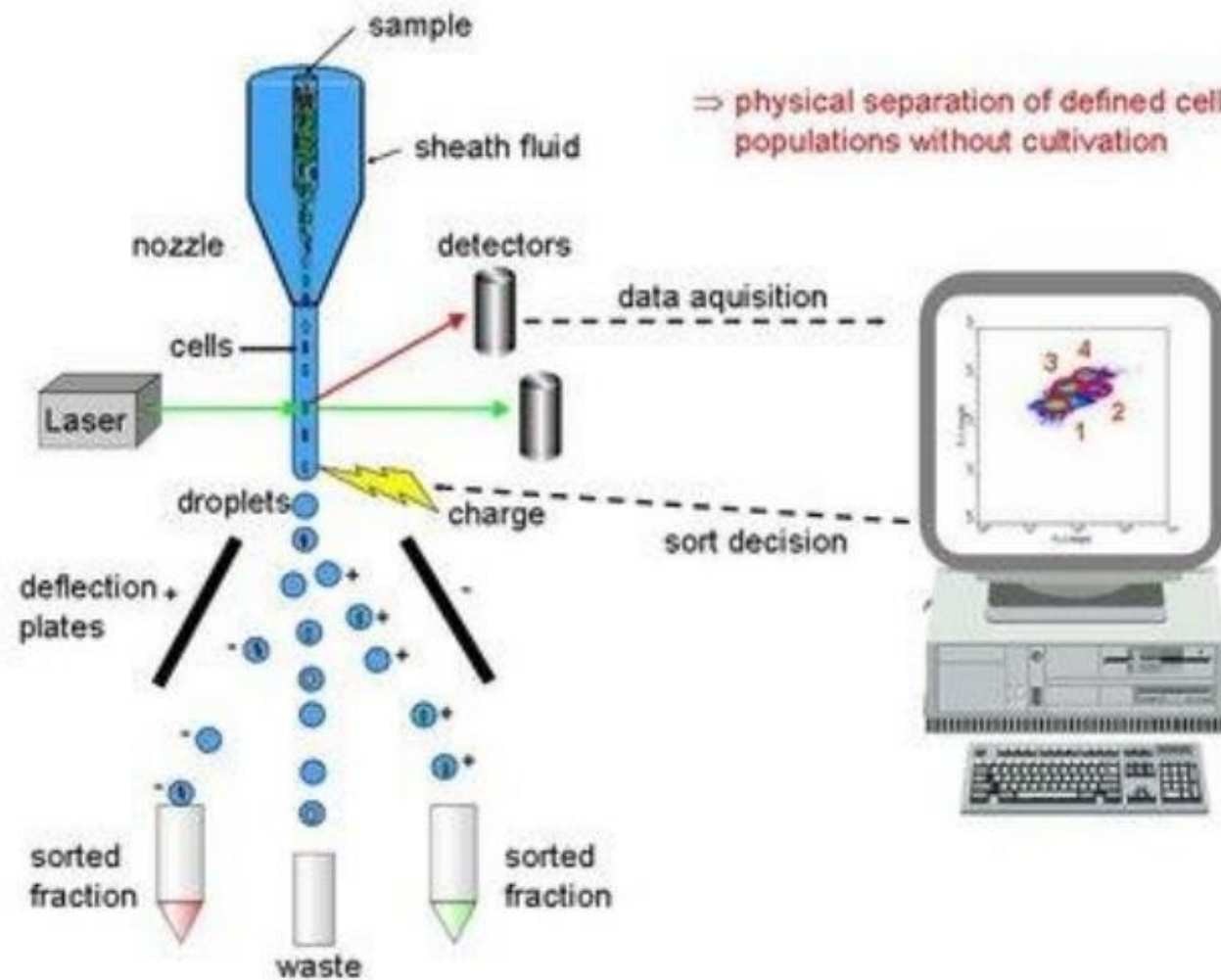


## Counting system to ensure accuracy and consistency



## ■ Flow cytometer

**Flow cytometry** is a laser-based, biophysical technology employed in cell counting, cell sorting, biomarker detection and protein engineering, by suspending cells in a stream of fluid and passing them by an electronic detection apparatus.





# 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



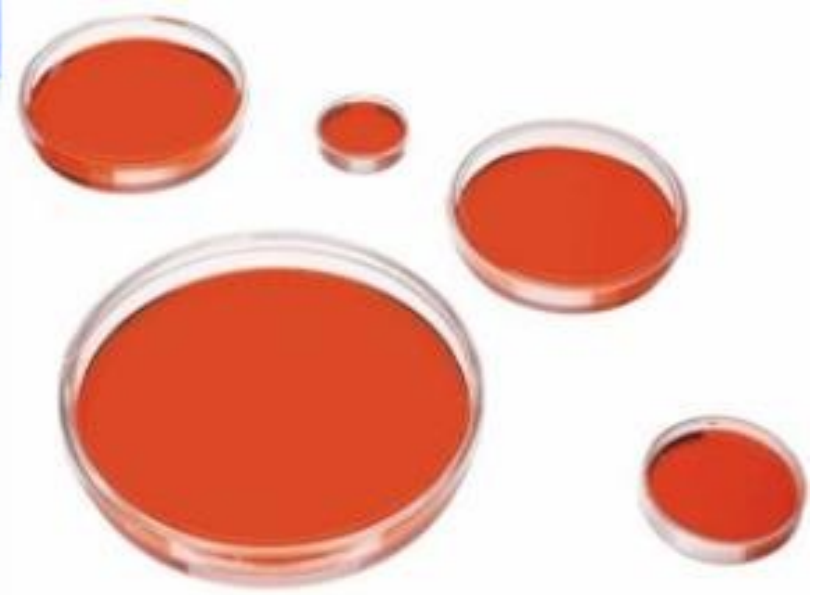
Description	Growth area (cm <sup>2</sup> )	Recommended working volume (mL)	Cell yield*
T-25	25	5 – 10	2.5 x 10 <sup>6</sup>
T-75	75	15 – 25	7.5 x 10 <sup>6</sup>
T-150	150	30 - 50	15 x 10 <sup>6</sup>
T-175	175	35 - 60	17.5 x 10 <sup>6</sup>
T-225	225	45 - 75	22.5 x 10 <sup>6</sup>

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

# 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 <sup>2</sup> )	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<sup>2</sup>.



# Culture Vessels

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



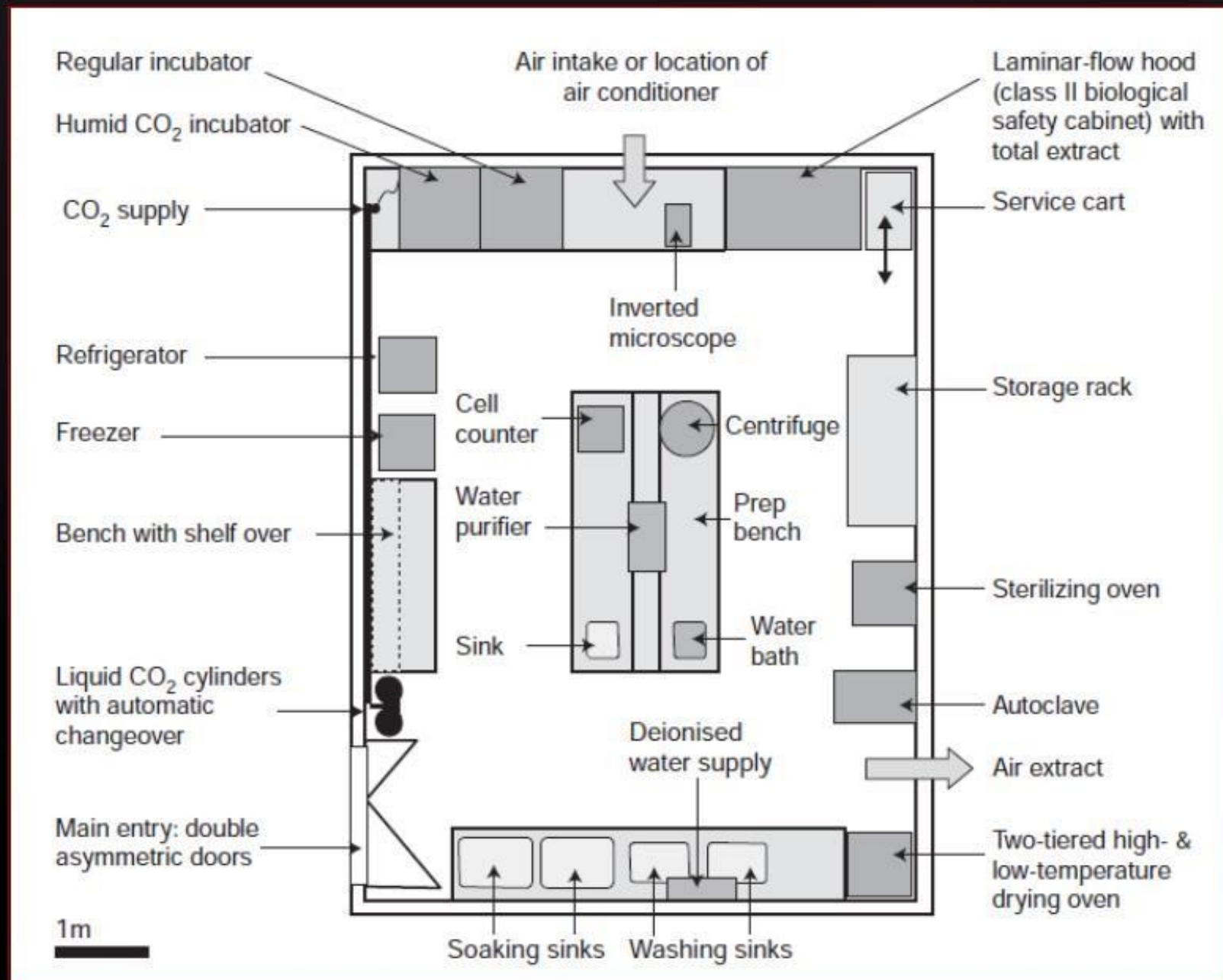
# Pipette aid







## Small Tissue Culture Laboratory suggested for use by two or three persons



# Tissue Culture Lab with Adjacent prep room with medium-sized tissue culture lab

