

Guidelines for use of Cell Culture Core




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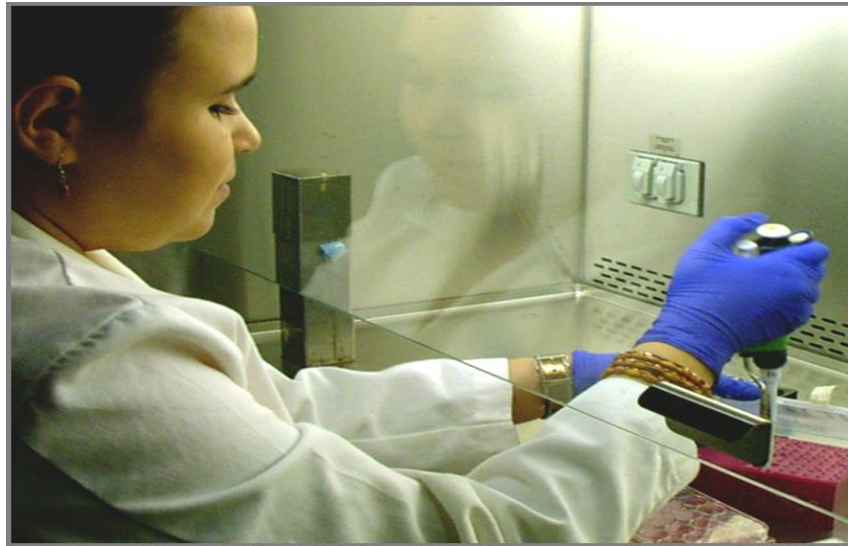


General rules for facility use

- Reagents and culture flasks must be labeled with your name, PI's initials and date.
 - Always sign equipment logs when using core equipment.
 - Do not share you access code.
 - Deposit waste in proper containers.
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Aseptic Technique

What is it?



Aseptic technique is everything you do to reduce the probability of contamination.

Important Pointers for Aseptic Techniques

- **Clean Work Area:** a clean hood and well organized work area.
 - **Personal Hygiene:** good hand washing habits , use of gloves and other personal protective equipment,(PPE) such as caps lab coats and face mask.
 - **Sterile handling:** Swabbing with 70% ethanol and capping
 - **Handling bottles and flasks;** culture flasks should be laid down horizontally and like bottles, held at an angle during manipulations. Never reach over any open container.
 - **Pipetting:** Use disposable plastic pipettes come in a convenient size range (e.g.. 1 ml, 5 ml ,10 ml and 25ml)
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Work area and equipment

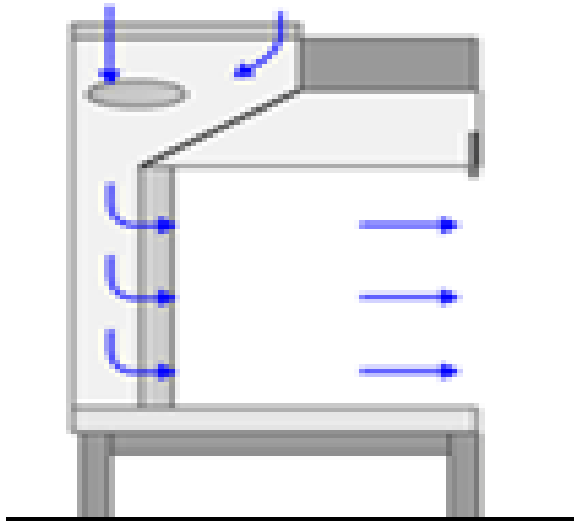
Biosafety Laminar air flow hoods

Types:

1. Horizontal air flow
2. Vertical air flow

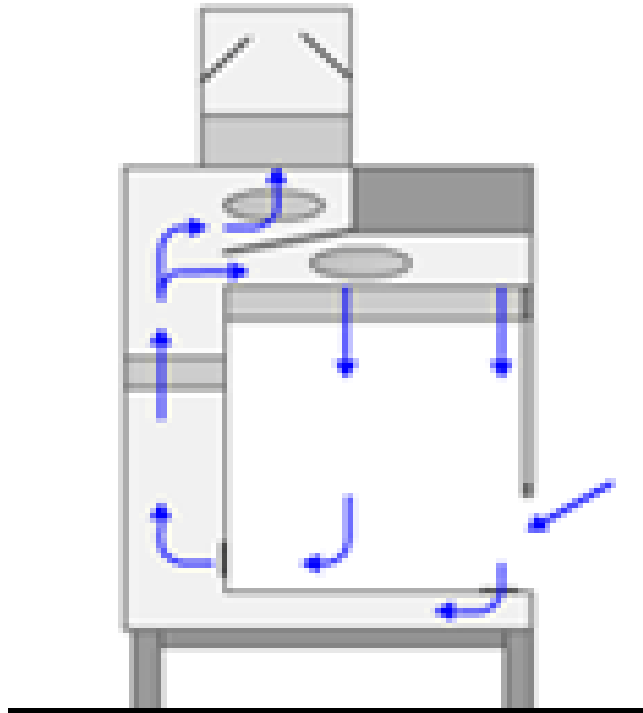


Horizontal



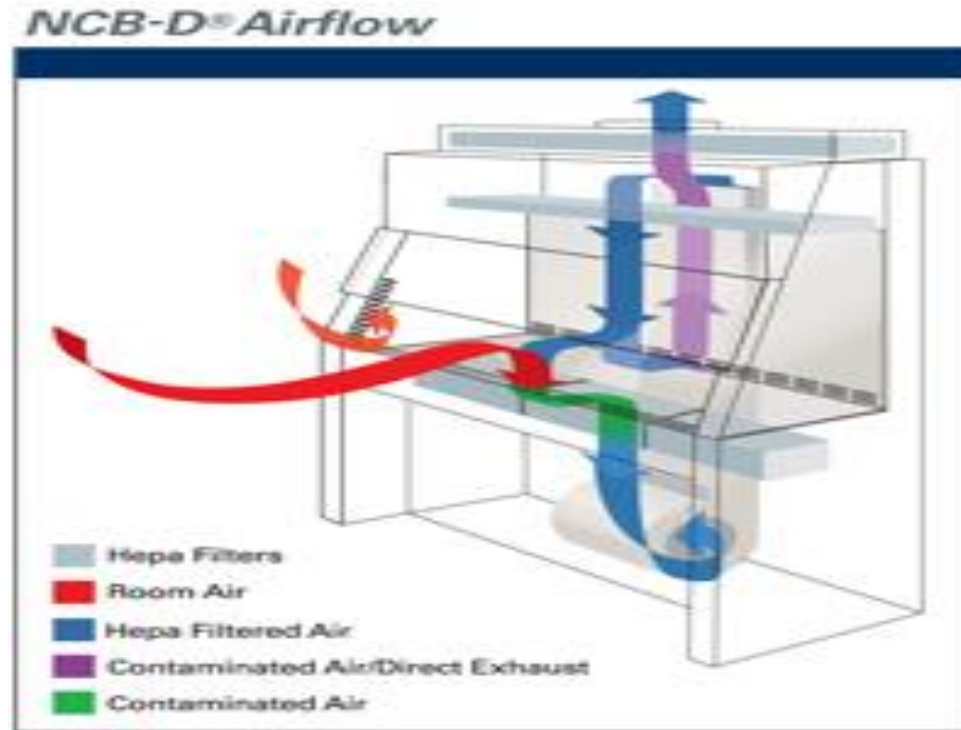
Horizontal air flow –used for media preparation and other sterile reagents and for culture of non primate cell lines. Provides no protection to user. Air flow is towards user

Vertical



Vertical air flow -used mainly for potentially hazardous materials (any primate, including human, cell lines, virus producing cultures, radioisotopes, carcinogenic and toxic drugs.) Usually equipped with UV light.

A Class II Vertical Type A2 laminar flow hood is the type used in cell culture



Incubators

- **For most mammalian cells**
 - Temperature at 37°C
 - Humidity at 95%
 - Carbon Dioxide at 5%

**Water is heated in a jacket surrounding the inner area to distribute heat evenly to the interior of the chamber
A water pan is placed with treated water to maintain humidity.**



Centrifuge

Cell culture procedures require the use of a refrigerated centrifuge



Inverted microscope

- Specially designed for viewing living cells
- Light source is above and phase objectives of 4X, 10X, 20X and 40X are located below.

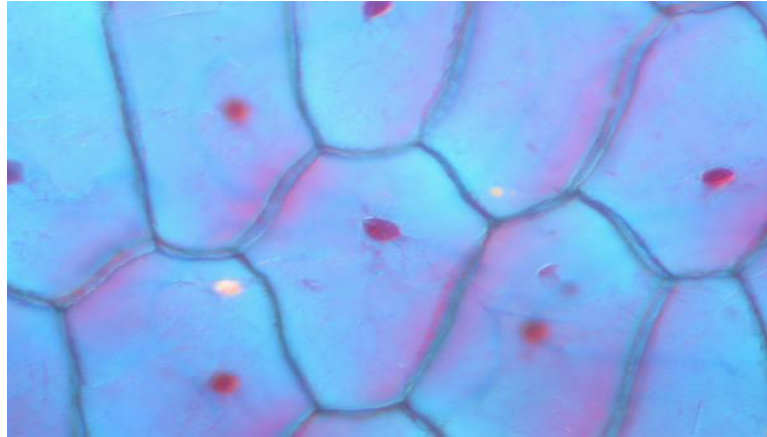


Equipment for Cryopreservation

The cell core facility has two ultra low temperature freezers (-140°C) and one (-80°C) low temperature freezer .




The importance of freezing cells



- Cross contamination of other cell lines
- Senescence and the resultant extinction of cell line
- Genetic drift due to genetic instability
- Contamination of microorganisms
- Need for distribution to other users

Basic safety considerations


- Minimum standard applied to any cell culture lab is category 2 containment.
 - Safety applies both ways;
 - protect your cells and protect yourself*
 - General safety rules
 - Aseptic vs. antiseptic technique
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Safety hazards of the cell culture lab

- Broken glass
- Electrical
- Radiation
- Frost bite
- Chemical toxicity
- Infection
- Biohazards



Basic safety procedures for cell culture

- Be aware of the potential for biohazard
 - Use of proper personal protective equipment (PPE).
 - Wash hands before handling cultures and before leaving the lab
 - Decontaminate work surface before and after use
 - Properly dispose of biological and chemical waste
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Quiz

Short answers please



1. What is the main advantage of good aseptic technique in the cell culture?
2. Give three examples of basic equipment of a cell culture lab.
3. Why is the vertical laminar air flow hood better for cell culture than a horizontal air flow hood?
4. Name three things you can practice to reduce the probability of contamination.