

# **Animal Tissue Culture**

## **SQG 3242**

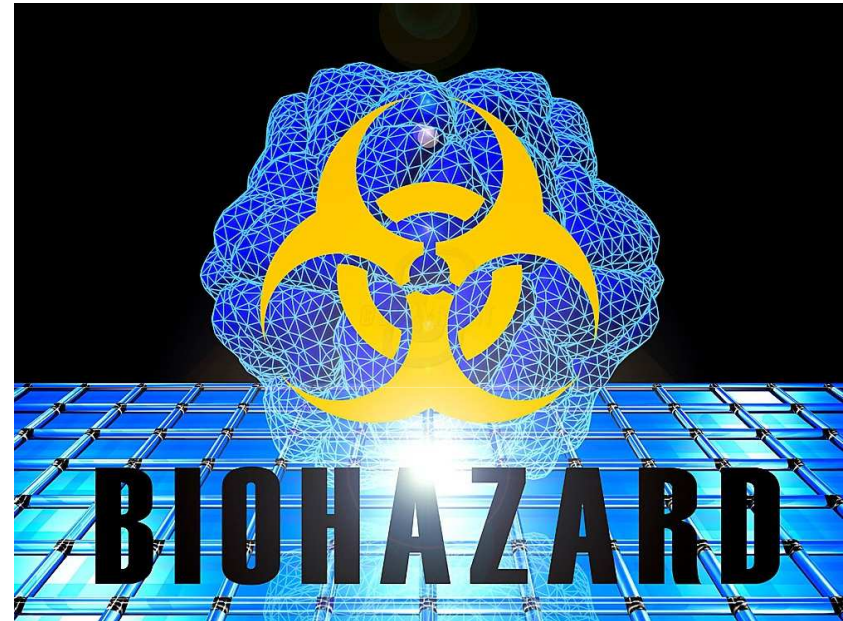
### **Equipment and Lab design**

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# Sign

- Symbol?
  - Biohazard sign
- Means?
  - Hazardous
  - For labeling the biological materials that carry a significant health risk, including viral samples and used
- Examples?
  - Virus or toxin samples, medical wastes



# Laminar flow cabinets

- Sterile environment
- Vertical or horizontal air flows
- Protect samples and/or worker
  - Class 1, 2 or 3
  - Sample>>>>worker

# What are Biological Safety Cabinets (BSC)?

Containment and protection devices used in laboratories working with biological agents with a primary purpose of protecting the laboratory worker and the environment from viable organisms.

# What is NOT a BSC!!!

- Chemical Fume Hoods
- Conventional lab fume hoods should NEVER be used to contain biological hazards.

Find a Biological Safety Cabinet!



# Class I Biological Safety Cabinets

- Class I BSC is a ventilated cabinet with an inward airflow and HEPA filters at its outlets.
- It was previously referred to as the **CDC Hood** and served a valuable function in its time to protect personnel and the environment.
- Because it **offers no product protection**, it has been essentially obsolete for the past several decades.



# Class II Biological Safety Cabinets

- As biomedical researchers began to use sterile animal and cell tissue culture systems, BSC's utility needed to be **expanded from simply protecting the operator to protecting the product as well.**
- Class II BSC's are Laminar Flow Biological Safety Cabinets that **protect personnel, product, and environment.**
- They provide an **inward airflow to protect personnel**, a **downward flow of HEPA filtered air to the work area to protect the product**, and then **exhaust HEPA filtered air to protect the environment** from particulate and aerosol hazards.



# Class II Type A BSC

- Class II, Type A hoods are used to protect **personnel, product and environment** from biological aerosols and particulates.
- These hoods offer personnel protection through negative pressure airflow into the cabinet.
- To protect the product, the work area in the cabinet is continuously bathed with ultra-clean air provided by the supply HEPA filter.
- Approximately **70% of the air of each cycle is recirculated through this supply HEPA filter.**
- The remaining air is discharged from the hood through the exhaust HEPA filter, protecting the environment.
- Although not required, most Class II hoods have the capability of being vented to the outside.





# Class II Type B BSC

- The Class II, Type B cabinet originated when the National **Cancer Institute** designed the Type 2 (later-Type B) **biological safety cabinet**.
- It was created for **manipulations of minute quantities of hazardous chemicals such as carcinogens** when used in research with in vitro biological systems.
- Carcinogens used in cell culture or microbial systems require both biological and chemical containment.

# Class II BSC Modifications

Class II BSCs can be modified to accommodate special tasks:

- The **front sash can be modified by the manufacturer to accommodate a microscope**
- A rigid plate with arm holes can be added if needed.
- The work surface can be designed to accept a carboy, a centrifuge, or other equipment that requires containment
- **Good cabinet design, microbiological aerosol tracer testing** of the modification, and **appropriate certification** are required to ensure that the basic BSC systems operate properly after modification. Maximum containment potential is achieved only through strict adherence to proper practices and procedures.



# Class III BSC or Glovebox

- The Class III BSC was designed for work with Biosafety Level 4 microbiological agents and **provides maximum protection to the environment and the worker.**
- It is a **gas-tight enclosure** with a **non-opening view window.**
- Access for passage of materials into the cabinet is through a dunk tank that is accessible through the cabinet floor or double door pass-through box such as an autoclave that can be decontaminated between uses.
- Reversing that process allows for safe removal of materials from the Class III BSC.
- Both supply and exhaust air are HEPA filtered. Exhaust air must pass through two HEPA filters or a HEPA filter and an air incinerator before discharge to the outdoors.
- **Airflow is maintained by a dedicated independent exhaust system exterior to the cabinet which keeps the cabinet under negative pressure**



# Class III BSC or Glovebox

Long, **heavy-duty rubber gloves** are attached in a gas-tight manner to ports in the cabinet and allow for manipulation of the materials isolated inside. Although these gloves restrict movement, they **prevent the user's direct contact with the hazardous materials**. The trade-off is clearly on the side of maximizing personal safety. Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment.



# Safe Work Practices for BSC Use

- **Do not store** equipment or supplies inside the cabinet.
- Do not use the **top of the cabinet** for storage. The HEPA filter could be damaged and the airflow disrupted.
- Make sure the cabinet is **level**. If the cabinet base is uneven, airflow can be **affected**.
- **Never disengage the alarm**. It indicates improper airflow and reduced performance which may endanger the researcher or the experiment.
- **Never completely close the window sash** with the motor running as this condition may cause motor burnout.
- **Cabinets should be placed** away from doors, windows, vents or high traffic areas to reduce air turbulence.

# Safe Work Practices for BSC Use

- For BSC **without fixed exhaust**, the cabinet exhaust should have a **twelve inch clearance from the ceiling** for proper exhaust air flow. Also, allow a twelve inch clearance on both sides of the cabinet for maintenance purposes.
- Never operate a cabinet while a **warning light or alarm is on**.
- The operator should be **seated with shoulders level** with the bottom of the sash.
- Perform all work using a **limited number of slow movements**, as quick movements disrupt the air barrier. Try to **minimize** entering and exiting your arms from the cabinet, but if you need to, do it directly, straight out and slowly.
- Keep all materials at least **four inches** inside the sash opening.
- To avoid excessive movements in and out of the cabinet, discard pipettes into a tray, container or biohazard bag within the cabinet.



# Safe Work Practices for BSC Use

- If a **bunsen burner** must be used, place it at the **rear of the work area** where the air turbulence from the flame will have the least possible effect on the air stream. Often the use of a flame is redundant in what should be a germ free work space.
- All equipment which has come in contact with the biological agent should be **decontaminated**. The cabinet should be **allowed to run for at least three minutes** with no activity so that the airborne contaminants will be purged from the work area before removing equipment.
- After all items have been removed, **wipe** the interior surfaces with disinfectant.

# Biohazard Spill Control Inside a BSC

- 1. Keep the BSC **on**.
- 2. Put on protective **gloves**.
- 3. **Spray & wipe** walls, work surfaces, and equipment with decontamination solution.
- 4. Flood tray top, drain pans, and catch basins with **decontamination solution**.
- 5. Allow to stand for **20 minutes**.
- 6. **Drain** excess solution into cabinet base.
- 7. **Lift out** tray and any removable exhaust grille work.

# Biohazard Spill Control Inside a BSC

- 8. **Clean** top and bottom surfaces with sponge/cloth soaked in decontamination solution.
- 9. **Replace** tray and grille work.
- 10. Place everything that is **contaminated** into autoclave pan.
- 11. **Drain decontamination** solution from cabinet base into AUTOCLAVABLE containers.
- 12. **Autoclave.**
- 13. If **gaseous decontamination** is needed, call EHS at 292-1284.

# Biological Safety Cabinet Certification

- Your cabinet must be **certified** when first installed and then annually.
- It must be **recertified anytime it is moved** even within the same room.
- Before certification personnel arrive, remove all items from the cabinet and wipe it down with a disinfectant.
- This will expedite the certification.
- If you have any questions, or think there may be a problem with your cabinet, do not hesitate to contact EHS (292-1284).
- Any decontaminations, certifications, repairs or adjustments are to be made by qualified personnel.

# And remember.....

- A laminar flow biological safety cabinet is a valuable supplement to good sterile technique.
- If the cabinet is not well understood and operated correctly, it will not provide adequate protection for you or the environment.

# References

- ▶ Freshney, R.I. (2000) Culture of Animal Cells: A manual of Basic Technique. 5fifth edition. New Jersey: John-Wiley & Sons, Inc.
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- ▶ Bernhard, O., & Bhatia, N. (2004) Tissue Engineering. Pearson Prentice Hall Bioengineering

