Equipment Safety Procedure

Biosafety Cabinets

All Biological Safety Cabinets (BSC) that are used with infectious materials must be certified annually and after repairs/being moved/maintained. Certifications are performed by EHS and repairs are performed by a contractor coordinated through EHS.

Purpose:

- Product, personal, and environmental protection
- Operating procedures
- Load BSC with all needed supplies
- Turn BSC on and allow to run for 10-15 minutes
- Check inward airflow with a piece of tissue
- Enter straight into cabinet and perform work in a slow, methodical manner
- At the end of work, decontaminate all items to be taken out of cabinet
- Decontaminate interior of BSC
- Allow cabinet to run for 10-15 minutes before shutting off
- Review owner's manual for additional instructions

Safe Operation:

- Always enter straight into cabinet – no sweeping motions
- Place materials well within the cabinet – never on front or back grill
- Place waste container within cabinet
- Watch for disruption of laminar flow hood
- Decontaminate materials before removal of cabinet
- In general, not designed for chemical use
- May use for non-volatile toxic chemicals or low-level radioactive materials
- May use for minute amounts of volatile chemicals
- Ensure annual calibration
- Place all work materials into cabinet before starting
- Work from clean side to dirty side
- Take machine out of service if broken or unused

Cautions:

- Chemical may damage HEPA filter

- Volatile chemicals not retained by HEPA filter (exposure to personnel if not exhausted)
- BSC fans are not spark proof – chemical use may result in fire or explosion
- DO NOT use Bunsen burners or other open flames – they can affect airflow and damage HEPA filters

Chemical Fume Hoods

Fume hoods are local ventilation devices used to limit your exposure to hazardous fumes, vapors, gases, or dusts when handling chemicals.

All fume hoods on campus are either constant volume hoods or variable air volume (VAV) hoods.

Constant Volume Hoods

These hoods permit a stable air balance between the ventilation system and exhaust by incorporating a bypass feature. The bypass allows some air to flow through it instead of the hood opening. This helps to minimize the increase in the velocity, as the sash is lowered. The volume of air exhausted from the hood remains constant regardless of sash position

Variable Air Volume (VAV) Hoods

These hoods maintain constant face velocities by varying exhaust volumes in response to changes in sash position. Because only the amount of air needed to maintain the specified face velocity is pulled from the room, significant energy savings are possible when the sash is closed. Effective sash management (pull sash closed when not using hood) is necessary in order to see lowered energy consumption.

Also, all hoods are either conventional or low flow. Conventional hoods typically operate at a velocity of 100 fpm at an opening of 18 inches. Low flow hoods (which reduce the amount of air that has to be exhausted through the hood, which equates to energy savings) typically operate with a velocity of 75 fpm.

Preparing the Fume Hoods for Work:

- All personnel using the fume hood must be fully trained in its proper operation
- Check alarms and monitors to make sure they indicate proper operation
- Observe noise and air movement to indicate proper operation
- Confirm that the hood is working by holding a tissue or a lightweight paper, up to the opening of the hood. The paper should be pulled inward.
- Check the EHS hood inspection sticker to ensure the hood has been inspected within the past year (contact EHS if it has not)

WARNING: If the alarm sounds or the monitor lights indicate low flow:

- Stop working
- Turn off the equipment
- Close the sash
- Contact EHS and SSC

Working in the Fume Hood:

- Monitor the fume hood when performing ongoing or reactive experiments
- Place:
- experimental materials and equipment at least 6 inches back from the sash opening, preferably in the middle of the hood
- place large objects two to three inches above the work surface to allow air to flow underneath. This dramatically reduces the turbulence within the hood and increases its efficiency
- Keep rear baffle openings clear
- Keep papers, paper towels, work surface diapers, cardboard out of the hood. These combustible materials add to the fire risk and can be drawn into the hood's ventilation system.
- Do not place objects directly in front of a fume hood (such as refrigerators or lab coats hanging on the manual controls) as this can disrupt the airflow and draw contaminants out of the hood

- Do not use a fume hood as a storage cabinet for chemicals. Excessive storage of chemicals and other items will disrupt the designed airflow in the hood.
- When the hood is not in use, pull the sash all the way down. While personnel are working at the hood, pull down the sash as far as is practical. The sash is constructed of safety glass to protect users against fire, splashes, and explosions.
- Keep in mind that modifications made to a fume hood system, e.g., adding a snorkel or cutting a hole in the side of the hood, can render the entire system ineffective. Modifications (even minor) can NEVER be performed by research personnel.
- Minimize the amount of foot traffic immediately in front of a hood. Walking past hoods causes turbulence that can draw contaminants out of the hood and into the room.

After using the Fume Hood:

- Fully close the fume hood sash
- Put chemicals and other items back in their proper storage locations

Other Precautions:

- Do not put your head in the fume hood

- Properly cap, seal, or cover chemical containers when they are not being used
- Properly label all chemical and chemical waste containers

Cleaning the Fume Hood:

- Clean the interior and exterior surfaces and sash periodically and after spills using deionized water; then wipe the areas down with a soap solution and rinse.

NOTE: Personnel should wear appropriate PPE when cleaning the fume hood to protect themselves from chemicals

Alarms and Monitors:

- Laboratory fume hood alarms or monitors should never be turned off. If the alarm sounds or the monitor lights indicate low flow, work should be stopped, equipment turned off, and the sash closed. Lab personnel should leave the area if highly toxic or volatile chemicals are being used. Report all issues to EHS and SSC.

Standards:

- The face velocity, which is the air moving into the fume hood entrance or access opening, should be 100 fpm for standard fume hoods or 75 fpm for low flow hoods.
- No other types of exhaust can be connected to the fume hood exhaust system without a proper engineering assessment by SSC.

Fume Hood Testing:

- Fume Hoods are tested and certified by EHS annually and after maintenance work has been performed.
- If your fume hood has not been certified within the last year, or if you have questions regarding fume hoods, contact EHS.
- If the fume hood is not functioning or is in alarm, contact SSC.
- Fume Hoods are testing July of every year.

Centrifuges

Centrifuges can create aerosols and this must be considered with each use. The necessary precautions taken will depend upon what is being used. If hazardous materials such as carcinogens, highly toxic, or infectious agents will be placed in a centrifuge, then precautions must be taken to prevent an exposure of lab personnel to aerosols or liquids.

Improper care of a centrifuge and its components can lead to exposure to hazardous substances, injuries, damaged equipment and lab space, and a loss of research, money, and time.

Types:

- Microcentrifuge ~ 15,000 rpm
- Low/High speed 2,000-20,000 rpm
- Ultracentrifuges ~ 120,000 rpm

Hazards:

- Mechanical failure of the machine
- Lab equipment failure
- Aerosol generation
- Operator Error

Operating Procedures:

- Follow all of the manufacturer's instructions for care
- Develop and implement a strict cleaning, storing, and maintenance protocol
- Only use manufacturer compatible centrifuge, rotor, buckets, caps, and adapters
- Replace centrifuge parts including bottles, tubes, and O-rings at the first sign of damage
- Assemble bottles, buckets, and rotor per the manufacturer's instructions
- Tightly seal all tubes and safety cups
- Close lid during operation
- Allow to come to complete stop before opening

Safe Operation:

- Use safety cups whenever possible.
- Disinfect or clean weekly and after all spills or breakages.
- Lubricate O-rings and rotor threads weekly.
- Do not operate the centrifuge without the rotor properly balanced.
- Do not use rotors that have been dropped.
- Inspect the speed disk for sign of damage if using an ultra-speed unit and discontinue use if damaged.
- Inspect the rotor and tube cavities for signs of damage and discontinue use if damaged.
- If using a swinging bucket rotor, follow these additional workplace practice controls:
  - Ensure all metal buckets are in place.
  - Use matching buckets, caps, and adapters
  - Load symmetrical to axis of rotation and to pivotal axis within the manufacturer's recommended load tolerance.

- Ensure buckets are properly seated to the rotor and the rotor is properly attached to the centrifuge spindle.
- If using a fixed angle rotor, follow these additional workplace practice controls:
  - Tighten rotor lid correctly.
  - Properly install and attach rotor to spindle.
  - Gently pull up on the rotor to confirm rotor is attached.
- Contact your centrifuge representative for specific information.

Cryogens

A cryogen or cryogenic liquid is defined by the National Institute for Standards and Technology (NIST) as any liquid with a boiling point below 93K (-180°C or -240°F) at 1 atmosphere of pressure. This definition includes liquid nitrogen (LN2), liquid argon (LAr), liquid helium (LHe), liquid hydrogen (LH2), and liquid oxygen (LO2), among others. This definition does not include liquid propane, liquid butane, liquid acetylene, or liquefied natural gas (methane).

Liquid helium, liquid hydrogen and liquid oxygen present additional hazards that liquid nitrogen and liquid argon do not. Liquid nitrogen is the most frequently used cryogen on campus.

The following table summarizes the physical properties of common cryogens:

Cryogen	Boiling Point (1 atm) °C (°F)	Critical Pressure (psig)	Liquid Density (g/L)	Gas Density (27°C) (g/L)	Liquid-to-Gas Expansion Ratio	Type of Gas
Argon	-186(-303)	710	1402	1.63	860	Inert
Helium	-269(-452)	34	125	0.16	780	Inert
Hydrogen	-253(-423)	188	71	0.082	865	Flammable
Nitrogen	-196(-321)	492	808	2.25	710	Inert
Oxygen	-183(-297)	736	1410	1.4	875	Oxygen [a]
Methane	-161(-256)	673	425	0.72	650	Flammable

Although oxygen does not burn, it will support combustion. Oxygen-enriched atmospheres may lead to violent reactions, such as rapid combustion or explosions, with incompatible materials.

Before the beginning of any experiment or work related to cryogens, all personnel must be familiar with cryogens, equipment, and the system they will be using. It is recommended to create a Standard Operating Procedure (SOP) to include a description of the work being performed and have this available for all lab workers. Include procedural information regarding the use of equipment. The SOP should include the manufacturer's instructions regarding the use of and controls for commercially obtained cryogen equipment.

Liquid cryogens have an extremely low temperature. Contact with the cryogenic liquids causes not only frostbite or cryogenic burns but can also damage materials like water pipes, flooring, sinks, and electrical cables.

Cryogens are stored in confined containers such as pressurized dewars and cylinders. Cryogenic liquid storage at warm temperature for an extended time can cause flash vaporization and produce extremely high pressure. Therefore, it is important to evaluate the pressure relief valve, venting valve, rupture disk and integrity of the container at least every month or before using it. Cryogenic fluids have large liquid-to-gas expansion ratios:

- LN2 is approximately 696 to 1, (by volume)
- LHe is approximately 757 to 1
- LAr is approximately 847 to 1

These ratios mean that any accidental release or overflow of these cryogenic liquids will quickly boil into gas and create an asphyxiation hazard by displacing the oxygen content of the surrounding area. In the case of liquid argon and liquid nitrogen, the gas generated from malfunctioning equipment or spills will be cold and denser than ambient air. Even well-ventilated lab spaces that have pits or other low-lying areas could have the oxygen displaced by this cold, dense gas. Large volumes of cryogen liquid used in small laboratory spaces or in poorly ventilated areas increase the asphyxiation hazard. Oxygen deficiency monitors should be used in these areas.

The extremely low temperatures associated with cryogenic liquids can easily condense moisture from the air and cause ice formation. This ice can cause components or systems to malfunction (e.g., can plug vent lines and impede valve operation) or can damage piping systems. Carbon steel, metals, plastics, porcelain, and many other materials increase their brittleness in contact with cryogen and become susceptible to failure or malfunction. Therefore, always consult with the most appropriate reference when selecting materials for cryogenic applications.

In many situations LN2 and LHe2 can condense the surrounding air. The concentration of oxygen in the condensed air will increase and lead to oxygen enriched conditions. The concentration of oxygen can reach as high as 80% and amplify flammability and explosion hazards. Air should be prevented from condensing into liquid nitrogen by using loose-fitting stoppers or covers that allow for the venting of nitrogen boil-off gas.

Using cryogens in high ionizing radiation fields can generate ozone or nitrogen oxides. These compounds may cause a potential explosion hazard when the cryogen condenses oxygen from the atmosphere. The best control measure is to minimize the accumulation of oxygen coming into contact with the cryogen and to keep containers free of hydrocarbon contamination.

Other cryogenic liquids present specific hazards in addition to the above concerns. Examples include:

- **Liquid oxygen** presents **enhanced combustion** hazards.
- **Liquid hydrogen** presents additional hazards of **flammability and material embrittlement**
- **Liquid helium** presents additional hazards of **material embrittlement and solidification of air**

Personal Protective Equipment while working with cryogens:

Cryogen gloves are intended for handling very cold items and to protect against an accidental splash. They are not designed to be submerged in a cryogenic liquid. Cryogen gloves are usually made from a non-porous material and are different from heat resistant gloves. Never used heat resistant gloves to handle cryogen. Cryogen gloves are usually loose fitting and easy to remove quickly. Always inspect the cryogen gloves integrity before each use for rips, tears, or holes where cryogen could get inside.

Close-toed shoes (without mesh tops) are required when handling cryogens. Shoes should have an enclosed heel and entirely covered tops.

A lab coat is required to minimize skin contact. **Ear plugs** can be worn to protect the ear in case a cryogenic vial explodes. Wear **full length pants**.

Transportation of Cryogen Container:

Transportation of cryogenic liquids in elevators poses a potential asphyxiation and fire/explosion risk if workers become trapped in an elevator with a container of cryogen. Pressurized cryogen cylinders are strictly prohibited in passenger elevators. Always use a freight elevator to carry cryogenic containers and cylinders. In any situation, do not store or transport cryogen containing containers in the closed cabin or trunk of a vehicle. Cryogens should never be brought onto a bus or shuttle. Cryogenic liquid cylinders and dewars must be transported in the open bed of a truck or similar vehicle. Do not use a personal vehicle to transport any cryogen container.

Small quantities of liquid nitrogen or liquid argon may be transported in and around buildings provided they are contained within an appropriate low pressure dewar (fitted with a lid) and carried by their handles. Do not transport cryogenic liquids in open dewars. Do not attempt to carry additional items while holding the dewar, such as books, beverages, samples or tools.

Larger, low-pressure dewars may come with casters/wheels attached to the bottom. Otherwise, the dewar may need to be secured to a dolly or cart. The dewar should be kept as low to the ground as possible when using a dolly or cart for transportation. Do not transport low-pressure dewars on the top shelf of a hand cart. Check the entire route ahead of time to ensure that it is free of any obstructions or obstacles that may cause a cart or the dewar to tip.

Heavy cryogenic liquid cylinders must be transported with an appropriate cart designed for the specific cylinder model. Never roll cryogenic liquid cylinders. Appropriate PPE is still required when transporting cryogenic liquids around buildings. Before transporting cryogenic liquid cylinders, the user must ensure that all process valves (gas supply, liquid supply, pressure builder, and vent) are closed.