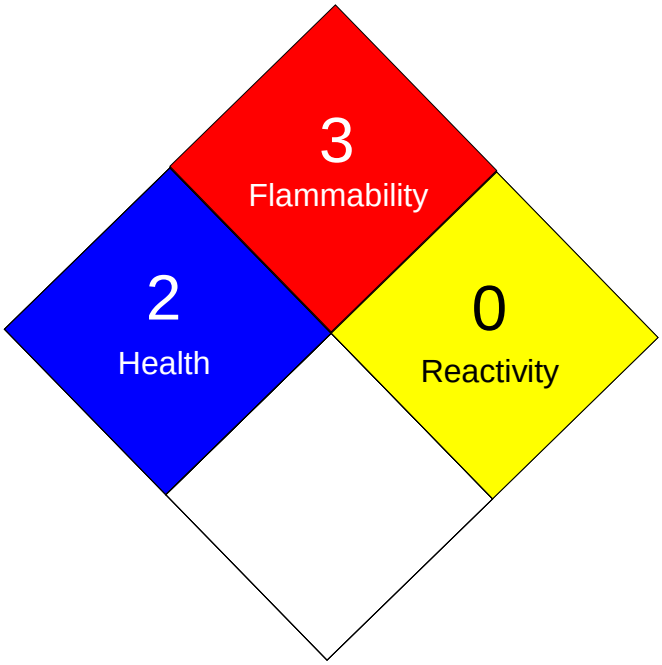
WVU Share Research Facilities

BNRF Lab
Chemical Hygiene Plan

G75E Engineering Science Building



Emergency Contact:

Huiyuan Li, Ph.D.
Office: 381 Chemical Research Laboratory building
Cell Phone: (304) 906-5368

SECTION 1: NFPA CHEMICAL HAZARD LABEL

NFPA Rating - The National Fire Protection Association (NFPA) has developed a standard system (ANSI/NFPA 704) for indicating the health, flammability, and reactivity hazards of chemicals. In addition, a special precaution symbol may be used where necessary.

This system of identifying hazards associated with various materials was developed primarily for fire protection and emergency personnel but can be useful to anyone who needs to handle potentially hazardous material. As stated in NFPA 704, "This standard provides a simple system of readily recognizable and easily understood markings, which will give at a glance a general idea of the inherent hazards of any material and the order of severity of these hazards as they relate to fire prevention, exposure, and control."

General Rating Summary:

Health (Blue)

0- Hazard no greater than ordinary material
1- May cause irritation; minimal residual injury
2- Intense or prolonged exposure may cause incapacitation: residual injury may occur if not treated
3- Exposure could cause serious injury even if treated
4- Exposure may cause death

Flammability (Red)

0- Will not burn
1- Must be preheated for ignition, flashpoint above 93°C (200°F)
2- Must be moderately heated for ignition flashpoint above 83°C (100°F)
3- Ignition may occur under most ambient conditions, flashpoint below 83°C (100°F)
4- Extremely flammable and will readily disperse through air under standard conditions, flashpoint below 83°C (100°F)

Instability (Yellow)

0- Stable
1- May become unstable at elevated temperatures and pressure, may be mildly water reactive
2- Unstable; may undergo violent decomposition, but will not detonate. May form explosive mixtures with water
3- Detonates with strong ignition source
4- Readily detonates

Special Symbols (White)

There are only two NFPA approved symbols although other symbols are also used to identify hazards:

OX- oxidizer
~~W~~- Water reactive, use no water
ACID - Indicates the presence of an acid in the lab – corrosive material with pH lower than 7.0
ALK - Denotes an alkaline material, also known called base. The caustic material has a pH greater than 7.0
COR - Corrosive material – it can be either an acid or a base.
LN2 - Liquid Nitrogen.

SECTION 1.1: EMERGENCY CONTACTS

In case of FIRE, INJURY, or EMERGENCY ASSISTANCE, contact in the following order:

9-911 from any campus phone
or
Campus Security
Phone: **(304) 293-3136 (293-COPS)**

Then Call

Huiyuan Li, Bio-Nano Research Facility Manager
Cell Phone: **(304) 906-5368**
Room: **381 CRL**
or

Kolin Brown
Cell Phone: **(304) 366-6551**
Office Phone: **(304) 293-9683**
Room: **G75D ESB**
or

Marcela Redigolo
Cell phone: **(304) 680-3007**
Office phone: **(304) 293-9973**
Room: **G75D ESB**

If no one responds to any numbers above, then contact:

Kenny Claudio
Cell Phone: **(304) 216-4858**
Office Phone: **(304) 293-4091**
Room**: 373A MRB**

Royce Watts,
Cell Phone: **(304) 288-6762**
Office Phone: **(304) 293-4124**

Room: **377A MRB**

For non-emergency assistance please contact:

Huiyuan Li, Bio-Nano Research Facility Manager
Cell Phone: **(304) 906-5368**
Room: **381 CRL**

SECTION 1.2: EMERGENCY EVACUATION PROCEDURES

In case of Fire or Chemical Spill all users should evacuate the laboratory immediately!

- Use the shortest, unobstructed path to the exterior of the building.

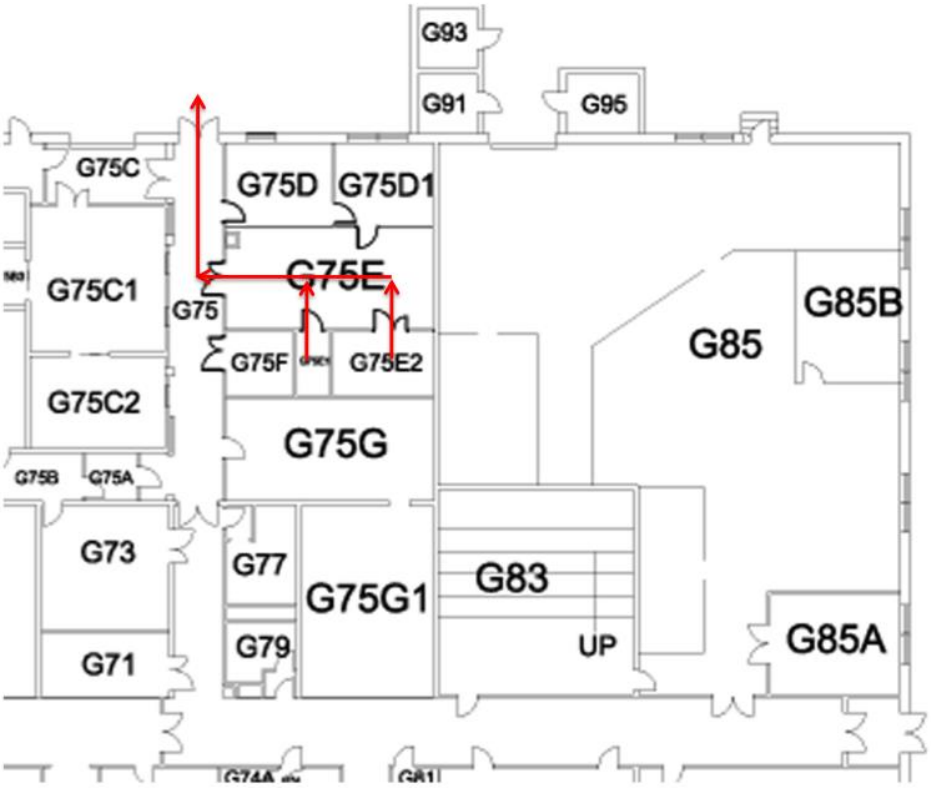


Figure 1: BNRF Lab Evacuation Plan for room G75E on the ground floor of the Engineering Sciences Building. Closest exits marked with red arrows.

- Evacuate the building by pulling the fire alarm pull station when exiting the building. All building exits have a fire alarm pull station.
  Do not wait next to the building. Move to a safe distance across the parking lot.
- Call the emergency contacts from a safe place.

SECTION 1.3: SHUTDOWN PROCEDURES

If a dangerous situation is evident (smoke, fire, sparks, etc.), ONLY if it is safe to do so, should a user attempt to shut down a system. The user should then notify all other persons in the lab to evacuate immediately. After evacuation, a user should contact proper emergency personnel from a safe place.

If no one is available and a machine is not acting as expected then the user should attempt to put the machine in its default mode; do not leave the machine running in an abnormal state! If the machine cannot be placed in its default mode, the user should stay by the tool and contact one of the WVU Shared Facilities Staff Members. If it becomes necessary to leave the tool then the user should leave a large, legible note on the machine stating that the tool is down, and the user's contact number.

Listed below are the procedures to place the BNRF ESB equipment into a safe default mode, or to shut down. Shutdown procedures may also be found on the cover of each instrument's logbook or in the Standard Operating Procedures found in Appendix A.

To place the Biosafety Cabinet in default mode:

- **Lower the sash to close the laminar flow hood and turn off the light.**

To place the Autoclave in default mode:

- **Press the cancel button on the LED screen. The current run will stop immediately however you will not be able to open the loading door until the temperature and pressure reach near ambient levels.**
- **If the run is finished, press the logoff button.**

To shut down the Autoclave (this emergency shutdown will turn OFF power):

- **Turn OFF the electrical breaker box, located at the wall to the left side of the autoclave.**

To shut down the Millipore water purification system and water supply:

- **Unplug the Millipore water system. Switch OFF the water supply at the wall to the right side of the Millipore.**

SECTION 1.4: EMERGENCY RESPONSE EQUIPMENT

First Aid kit is located inside the lab, **G75E**, attached to the wall by the entrance door.

The nearest **AED** (Emergency Defibrillator) is located in the **MRB Atrium**. A second AED can be found on the 1st floor lobby of the Engineering Sciences Building (ESB).

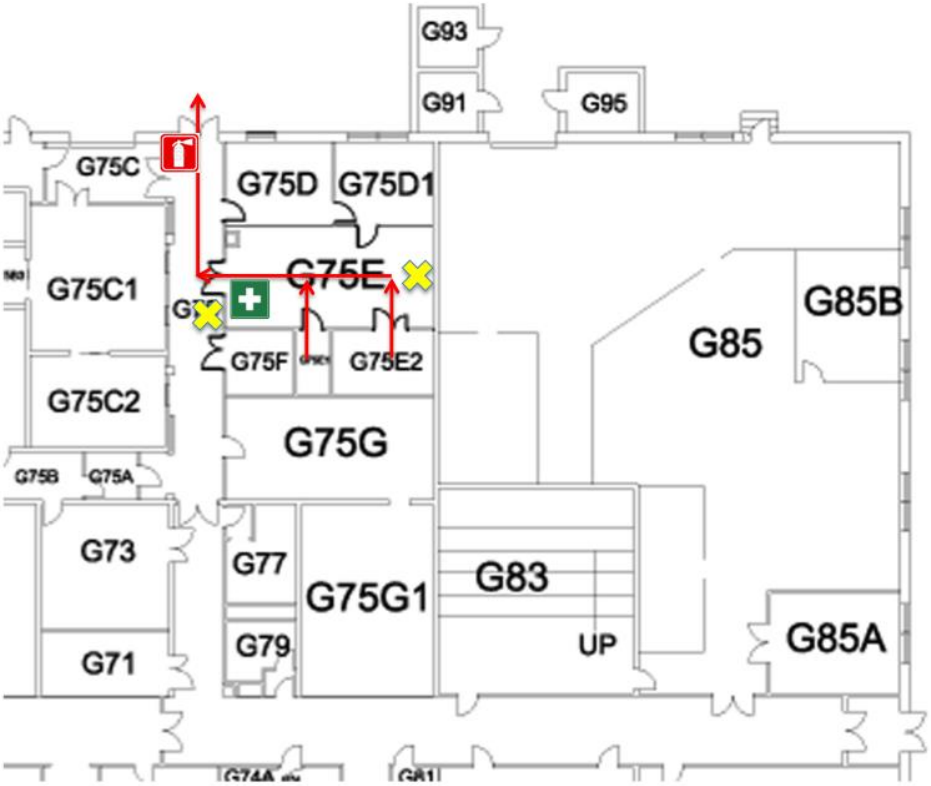


Figure 2: Location of the safety showers ( ), eyewash stations ( ), BNRF lab first aid kit ( ) and fire extinguisher ( ).

A **safety shower** and **eyewash station** is located in the corridor outside the G75E in the ground floor of the Engineering Sciences Building (ESB). Another **eyewash station** is located inside G75E lab over the sink (under Millipore water purification system). Figure 2 indicates the location of these safety shower and eyewash station using symbol.

The **Chemical Hygiene Plan** and **SDS**s are located inside the lab by the entrance door, **G75E ESB**. A fire extinguisher, type ABC, is located at the end of the corridor, near Exit of ESB, indicated in figure 2 by the  symbol.

A copy of part of this Chemical Hygiene Plan and related SDSs for the BioSample Prep Lab can also be found in G75E2 ESB.

SECTION 2: LAB OVERVIEW

The Bio-Nano Research Facility (BNRF) Laboratory in the Engineering Sciences Building (ESB) is one of two BNRF labs. The second is located in the Chemistry Research Laboratories (CRL) Building on the downtown campus. The BNRF is one of the five WVU Shared Research Facilities (WVUSRF). As part of the WVU Shared Research Facilities, the BNRF lab provides student and postdoctoral researchers with the opportunity to learn how to use cutting-edge cell culture, imaging, and biosample identification and quantification equipment. The facility is open to all researchers, including researchers at University, government laboratories and industries.

SECTION 2.1: FACILITY DESCRIPTION

The BNRF lab in the ESB is located in the main room G75E and contains two other smaller rooms within its space, rooms G75E1 and G75E2. The only equipment located inside room G75E1 is the autoclave.

Room G75E2 is the BioSample Preparation Lab for Electron Microscopy, under responsibility of the WVURSF Electron Microscopy Facility (EMF). This chemical hygiene plan describes hazards and procedures that are valid for all labs within G75E, except when stated otherwise. Specific hazards related only to room G75E2 will be described clearly in a separate section of this document.

The BNRF contains biohazard material in addition to safety hazards from contained chemicals and equipment.

SECTION 2.2: LABORATORY ACCESS

Access to the WVU Shared Research Facilities is controlled through the WVU ONITY Lock system. The door to G75E ESB has one ONITY lock. G75E1 and G75E2 do not have locks.

The BNRF Onity lock is subject to the CEMR lock policy. A version of the CEMR Electronic Lock policy may be found on the website http://www.its.cemr.wvu.edu/cardlocks/index.php or a full, written version may be obtained upon request to the Dean's office.

The following persons are the official operators of this lock:

- Kolin S. Brown
- Lisa Sharpe
- Harley Hart
- Royce Watts

In addition, the WVU Lock Shop and WVU Card Services also have capabilities to add students, staff and faculty to this lock.

SRF users are only given access to the BNRF laboratory during normal working hours after they have completed all required safety trainings and have a signed user agreement on file with the SRF Facility Manager.

The normal working hours of the lab are 8 AM to 5 PM, Monday to Friday. After-hours are 5 PM to 8 AM, Monday to Friday, and full time during weekends and holidays.

SECTION 2.3.1: REQUIRED SAFETY TRAINING

All users must complete the following safety trainings to receive access to the facility during normal working hours.

- SRF General Lab Safety Training
- SRF Chemical Safety Training
- Biosafety Training

Copies of the safety training presentation slides are located on the Shared Research Facilities website:
http://sharedresearchfacilities.wvu.edu/info/safety/

SECTION 2.3.2: USER AGREEMENTS

All SRF users must have a user agreement on file with the WVUSRF BNRF and/or EMF Manager. User agreements must be signed by the user, the user's advisor/supervisor and the user's department chair, when appropriate.

Copies of the User Agreements are located on the Shared Research Facilities website: http://sharedresearchfacilities.wvu.edu/r/download/160912

SECTION 2.3.3: DRESS CODE

The following dress code is required for all users entering G75E Lab:

1. No shorts, legs must be fully covered
2. No sandals or open toed shoes, feet must be fully covered
3. Long hair should be tied back.

Users operating cell culture and sample preparations must wear proper protection gear (listed below) and adhere by accepted safety protocols:

- Nitrile gloves
- Lab coat

Users operating autoclave must wear proper protection gear (listed below) and adhere by accepted safety protocols:

- Heat Insulated gloves

Users working with cryogenics must wear proper protection gear (listed below) and adhere by accepted safety protocols:

- Cryogloves (Tempshield)

Users working in room G75E2 must wear at all times:

- Nitrile gloves
- Lab coat
- Splash goggles

SECTION 2.3.4: AFTER HOUR ACCESS

After hour access to G75E Lab is given upon request by the WVU Shared Research Facilities BNRF and/or EMF Manager. The managers will use their own discretion to grant access when a user has demonstrated that he or she can work alone safely and handle an emergency.

SECTION 2.3.5: TEMPORARY USER SUPERVISION

Temporary users in the G75E lab must be accompanied by a trained user or staff member at all times. Temporary users may include summer research participants, visitors or class participants.

SECTION 3: LABORATORY HAZARDS

Users should be aware of the following safety hazards when working in BNRF lab:

- Biohazards
- Thermal burns
- UV light exposure
- Cryogenics
- Mechanical hazards

Additional hazards, including chemical hazards that only apply to room G75E2 are described in a separate section.

SECTION 3.1: BIOHAZARDS

NOTE: This manual describes biological safety cabinet safety and disposal of biohazards. All persons entering this lab must have completed Biosafety Training in compliance with WVU EH&S and CDC guidelines. This DOES NOT cover best biological practices and protocols.

Any areas, equipment and materials (including wastes) that present a biohazard must be clearly identified by an appropriate label or sign. An example biohazard sign is shown in Figure 3.

Figure 3: Example biohazard label

Biological safety cabinets (BSCs) are primary devices intended to contain and minimize exposure when working with biohazardous materials. BSCs are designed to protect laboratory personnel against exposure during experimental procedures as well as protect experimental materials from contamination. BSCs utilize vertical laminar airflow to achieve a barrier of protection against airborne contaminants. The laminar airflow reduces turbulence in the work area and minimizes cross contamination. The BSCs in the BNRF are Thermo Scientific 1300 Series A2 units and contain HEPA (High Efficiency Particulate Air) filters to create a near sterile working environment. The BSCs are also equipped with ultraviolet (UV) for germicidal applications. Care must be used to always have the sash down while the UV light is on to prevent exposure to UV radiation. BSCs are inspected and certified annually for proper airflow and filter integrity to ensure they are functioning properly.

Using the BSC is required in BNRF when handling the following materials or procedures:

- Culturing cells (including seeding cells, changing media, splitting cells, and preforming assays, etc)
- Preparing culture media or other liquids using for cells
- Removing caps from tubes after centrifugation, vortexing open tubes, aspirating with syringe, etc.

Use of a BSC must be used in combination with aseptic technique (such as decontamination with 70% ethanol, and material sterilization with autoclave, etc) to ensure proper protection from exposure to infections materials.

All refrigerators and freezers within the lab are for sample and chemical storage.

DO NOT store any food or drink in any of the refrigerators or freezers in the laboratory. (Food and drink are not permitted in the lab at any time!)

SECTION 3.1.1: BIOLOGICAL WASTE DISPOSAL

The following wastes generated in the lab are considered biological waste:

- Cell culture waste liquid
- All sharps
- Plastic serological pipettes, tips and cell culture vessels

The following procedures must be used for disposal of:

1. Liquids — Cell culture waste liquids are aspirated into the vacuum flasks containing bleach. When the flask is half full, add more bleach. After sitting 30 min with bleach, pour down the drain. Wash the flask with tape water. Then decontaminate with 70% ethanol and wipe the outside surface with paper towel.
2. Sharps — Sharps (needles, glass Pasteur pipettes, etc.) should be disposed in the sharp container located on the workbench. SRF staff will seal the sharp container and autoclave it and then dispose as regular trash.
3. Lab vessels — Plastic serological pipettes, tips, cell culture vessels should be disposed in the biohazard bag in the drum under the biosafety cabinet. SRF staff will autoclave the biohazard trash when it reaches 2/3 full. Then it will be sealed and disposed as regular trash.

SECTION 3.1.2: BIOLOGICAL LIQUID SPILL

The following procedure should be followed when the biological liquid spill happens in BNRF lab.

1. Spills on the lab floor or bench:

- Alert personnel in vicinity to leave the immediate area.
- Wear personal protective equipment (gloves, goggles, and lab coat).
- Cover an area twice the size of the spill with disinfectant (i.e. 70% ethanol or 10% bleach) soaked paper towels.
- Pour additional disinfectant solution onto the spill. Avoid splashing.
- Allow 20 minutes contact period.
- Wipe down any contaminated stationary equipment or furniture twice with disinfectant.
- Use forceps to remove broken glass and other sharp items, and place in sharps container.
- Remove towels and re-clean area with disinfectant solution.
- Collect and dispose the paper towel in regular trash container.
- Decontaminate (autoclave, or use a chemical disinfectant) reusable clean-up items and other permanent equipment.
- Inform laboratory personnel when the clean-up is complete.

2. Spills inside the Biological safety cabinet (BSC)

- Keep the cabinet running.
- Clean up the spill area with disinfectant (i.e. 70% ethanol or 10% bleach) soaked paper towels similar as the description above.
- If the material has spilled on the pipette tips, wipe the tips with disinfectant soaked paper towel and disposal the tips in the sharp container.
- If the material has spilled on the small instrument, such as the pipetter, wipe twice with disinfectant soaked paper towel. And leave it in BSC.
- If the material has spilled in the grill panel, notify the lab manager, and the trained technician will handle it during service.
- Wipe down working surface, side wall, and items inside the BSC once more with towels and disinfectant.
- Remove all used paper towels into regular trash container.
- Place all the re-useable items in autoclave pan. BNRF staff will autoclave items.
- Place all the broken sharps in sharp container.
- The BSC must run for at least ten minutes after cleanup before being used for experiments.
- Report the spill incident to BNRF staff.

SECTION 3.2: THERMAL HAZARDS

Thermal burns can occur within the BNRF when working with the following equipment:

- Autoclave (G75E1)

- Hot plates (G75E)
- Dry heat block (G75E)
- Ovens (G75E2)

All lab users are given proper instruction on equipment operation before given authorization to use the tool. Users must follow all operational procedures outlined in the training and in the Standard Operating Procedures located in Appendix B.

Users must wear heat-insulated gloves to operate the autoclave. It is also suggested that the heated insulted gloves be worn when operating hot plates or the dry heat block with high temperature.

If you are burned, you should seek medical treatment immediately. Severe burns to the face, third-degree burns, or burns over large areas of the body should be treated as emergencies. Report the burn to our staff and your supervisor as an occupational injury.

Minor burns should be treated by using first aid procedures. Immerse the burn in cool water immediately. Remove clothing from the burn area, and keep the injured area cool for at least five minutes.

SECTION 3.3 UV LIGHT EXPOSURE

UV light exposure can occur from the following equipment:

- Biosafety cabinet (main room G75E)
- EVOS FL (room G75E2)

All lab users are given proper instruction on equipment operation before given authorization to use the tool. Users must follow all operational procedures outlined in the training and in the Standard Operating Procedures located in Appendix B.

UV light sources are used in the biological safety cabinet and fluorescence microscope. There are three ranges of UV radiation. UV-A (320-400 nm) has lowest hazard potential. UV-B (290-320 nm) has mid to high hazard potential, and can cause skin and eye burns. UV-C (190-290 nm) has the strongest hazard potential causing severe burns. The severity of the UV hazard is elevated by the fact that UV radiation symptoms of overexposure are not immediately felt. Therefore special care should be taken for UV exposure.

UV-C (254 nm) is commonly used in Laminar air flow cabinet (biosafety cabinet). This should be treated with extreme caution. DO NOT expose yourself to BSC UV light. **The UV lamp should never be left on when the hood is open**. Even a small opening at the bottom of the cabinet can exceed occupational exposure standard several feet away.

UV light usage in biosafety cabinet:

- Before starting experiment, keep the sash off, TURN ON the UV light, and keep it on for 15 minutes. Then TURN OFF UV light. Open the sash. Clean the surface of the BSC. Then start the experiment.
- When experiment done, clean the BSC. Close the front window of the BSC. Then TURN ON the UV light. The UV light will switch off automatically after one hour.

Fluorescence microscopy uses UV light source. The UV beam is sent through the objective to the sample. The emission light is fluoresced light, not the UV light. This is considered safe. However, when you position you sample slides, you might look directly at UV light range. It is mandatory to wear UV protection goggles while working with the Fluorescent microscope. The goggles can be found by the side of the microscope. So when the user adjusts sample slide position, the following safe procedure is required.

- Switch the light source back to “bright flied” and then adjust sample position, or use the mechanical stage control knob to move the sample.
- There is a removable plastic light cover which is used to cover the top the sample stage. This cover will block UV light exposure and is always required to use when the UV light source is ON.

Acute effects include redness or ulceration of the skin. The long-term large cumulative chronic exposure includes premature aging of the skin, wrinkles, and skin cancer. The eyes are also susceptible to UV damage. The danger to the eyes is enhanced by the fact that light can enter from all angles around the eye and not only in the direction where one is looking. Chronic exposure to UV-A radiation can lead to the formation of cataracts. When such incidents of overexposure occur, it is important to identify the causes and adjust your practice to prevent future incident, and seek medical treatment. In case of exposure, seek medical attention immediately.

SECTION 3.4: CRYOGENIC HANDLING

The BNRF has an ultralow temperature freezer for sample and reagent storage (-80 °C). Working with materials that have been stored in the freezer is dangerous and can cause cryogenic burns or damage to human skin.

- Waterproof thermal insulated gloves are required when working with the ultralow freezer.

SECTION 3.5: MECHANICAL HAZARDS

Room G75E has several centrifuges, mixers, and shakers for sample preparation. Some general safety guidelines must be followed:

- Do not wear loose fitting clothing and necklaces that could be drawn into a rotating assembly.
- Do not defeat interlocks on doors, access panels, etc.
- Do not break moving parts by hand. Wait for motion to stop on its own.
- Be aware of assemblies that vibrate and could "walk" into other objects or fall off the bench.
- Safety glasses must always be worn around any power tool (such as centrifuges, mixers, and shakers) when operating.
- Always be familiar with the operating procedures writing by the manufacturer.
- For centrifuge, pay careful attention to instructions on balancing samples. Check the condition of tubes and bottles. Make sure you have secured the lid to the rotor and the rotor to the centrifuge. **ALWAYS** use screw-capped tubes!

SECTION 4: GAS SAFETY

The following gas cylinders are typically used by cell culture incubator inside the cell culture room, G75E:

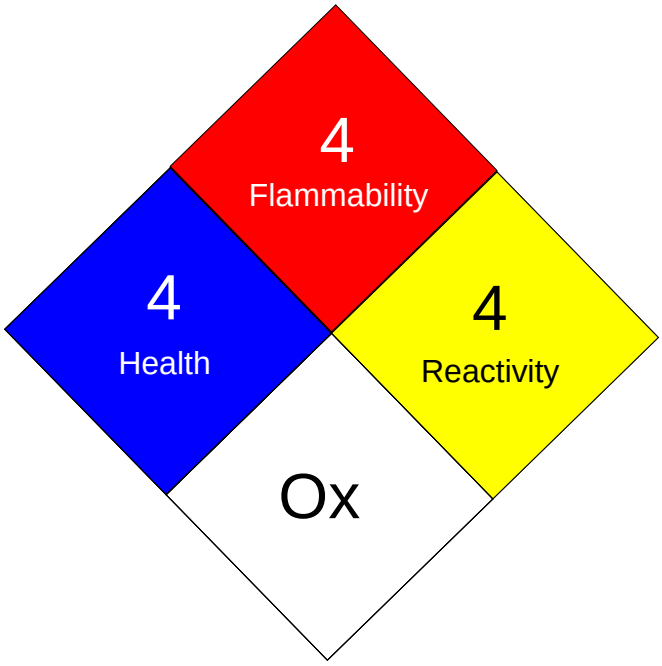
- Carbon Dioxide

Users should not have to open, close, or make adjustments with the regulators. Adjustments to regulators should be done by SRF BNRF staff.

All cylinders in use are mounted securely against a wall. All cylinders are transported using a cylinder hand truck. Eye protection or face shield is to be worn when changing cylinders.

SECTION 5: G75E2 - CHEMICAL HYGIENE PLAN

The following safety information is specifically for ESB G75E2.



Emergency Contact:

Marcela Redigolo, Electron Microscopy Facility Manager
Cell Phone: **(304) 680-3007**
Room: **G75D ESB**

In case of FIRE, INJURY, or EMERGENCY ASSISTANCE, contact in the following order:

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Huiyuan Li, BioNano Research Facility Manager
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Room: **CRL 381**
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SECTION 5.1: SHUTDOWN PROCEDURES

If a dangerous situation is evident (smoke, fire, sparks, etc.), ONLY if it is safe to do so, should a user attempt to shut down a system. The user should then notify all other persons in the lab to evacuate immediately. After evacuation, a user should contact proper emergency personnel from a safe place.

If no one is available and a machine is not acting as expected then the user should attempt to put the machine in its default mode; do not leave the machine running in an abnormal state! If the machine cannot be placed in its default mode, the user should stay by the tool and contact one of the WVU Shared Facilities Staff Members. If it becomes necessary to leave the tool then the user should leave a large, legible note on the machine stating that the tool is down, and the user's contact number.

Listed below are the procedures to place the BioSample Prep Lab equipment into a safe default mode, or to shut down. Shutdown procedures may also be found on the cover of each instrument's logbook or in the Standard Operating Procedures found in Appendix A.

To place the CO2 Critical Point Dryer in default mode or turn OFF the instrument:

- **Turn OFF the power switch located on the back of the instrument**
- **Turn OFF the controller power switch.**
- **Close the CO2 bone dry cylinder valve.**

To shutdown (this emergency shutdown will turn OFF power to the entire instrument):

- **Close the CO2 bone dry cylinder valve.**
- **Unplug the power cords from the wall.**

To place the Ultramicrotome in default mode:

- **Turn OFF the power switch on the back of the instrument**

To shutdown (this emergency shutdown will turn OFF power to the entire instrument):

- **Unplug power cord from the wall.**

To place the Furnace(s) in default mode:

- **Turn OFF the furnace by rotating the temperature knob to the Zero position.**

To shutdown (this emergency shutdown will turn OFF power to the entire instrument):

- **Unplug cable from the power outlet on the wall.**

SECTION 5.2: LAB OVERVIEW AND DESCRIPTION

The BioSample Prep Laboratory (G75E2) in the Engineering Sciences Building (ESB), is located inside the Bio-Nano Research Facility lab (G75E). While the BNRF is a different facility under the management of Dr. Huiyuan Li, the BioSample Prep Lab is part of the Electron Microscopy Facility (EMF) under management of Dr. Marcela Redigolo.

Both facilities are part of the WVU Shared Research Facilities (SRF), and provide students and postdoctoral researchers with the opportunity to learn how to use cutting-edge materials science and engineering equipment. The facilities are open to all researchers, including researchers at government laboratories and industries.

Because the BioSample Prep Lab is inside a BNRF space, any person working in the lab will need to follow all safety protocols and procedures requested by both labs, to ensure a safe working environment.

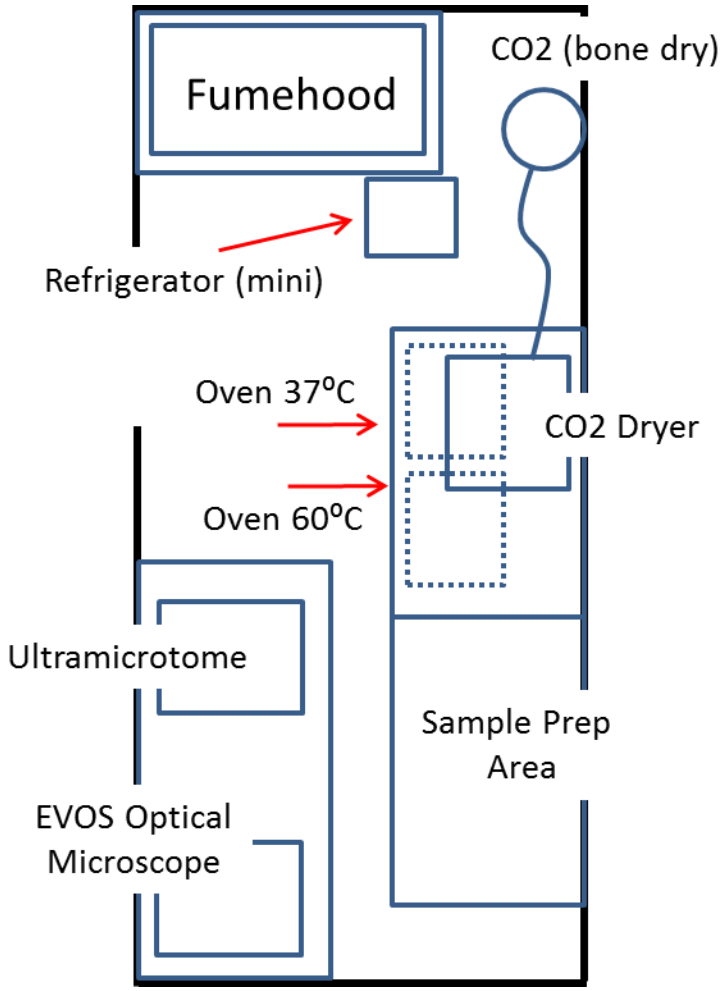

Figure 4: Representation of the instruments distribution within room G75E2.

The BioSample Prep Lab contains specific chemical and physical hazards related only to this room. They are addressed in this section of the Chemical Hygiene Plan. All hazards related to all rooms within lab G75E are addressed throughout this Chemical Hygiene Plan.

SECTION 5.3: CHEMICAL SAFETY

The following chemicals are always stocked in room G75E2:

- Acetone
- Ethanol
- Isopropanol
- Glutaraldehyde
- Dubecco's Phosphate Buffered Saline (PBS)
- Dodecany Succinic Anhydride (DDMS)

- Nidic Methyl Anhydride (NMA)
- ER-SCI Scipoxy 812 Rsh
- DMP-30 DiMethylAminomethyl
- Osmium Tetroxide
- Propylene Oxide

This list represents the standard chemicals used inside the BioSample Prep Lab. A current chemical inventory may be found in Appendix B. This inventory is updated yearly.

SECTION 5.3.1: CHEMICAL STORAGE

Chemicals in G75E2 are stored under the fume hood in its flammable cabinet or inside a minirefrigerator, depending on chemical requirements.

When a chemical order arrives, an approval chemical label that is dated and signed by a SRF staff member is applied to each chemical container. The chemical is then stored in the appropriated storage space.

The approved chemical labels are color coded to quickly identify proper chemical storage locations. The following color code is used:

- Green for acids and etchants
- Yellow for solvents
- Pink for polymers
- Blue for cell cultures
- Orange for bases

The minirefrigerator is used to store:

- Osmium Tetroxide
- Polymeric beads for TEM calibration
- Flammables cannot be stored in the minirefrigerator

NOTE: Osmium Tetroxide is stored within two enclosed and sealed containers, the external one serving as a secondary container. Only a small amount of the prepared solution of Osmium Tetroxide is stored in this lab.

SECTION 5.3.2: AUTHORIZING CHEMICAL USAGE

The WVUSRF Electron Microscopy Facility (EMF) only purchases chemicals that are general use. Often, research projects require the use of chemicals that are specific to a project. Any user, who wishes to bring a new chemical into the lab, must first obtain permission from the facility manager. The user must submit a signed material tracking form and a material safety data sheet(s) (MSDS) for each chemical container to the EMF Manager for approval. A copy of this

form can be found on the shared research facilities website (http://sharedresearchfacilities.wvu.edu/info/forms/). This form identifies the chemical, proper storage and proper disposal methods. Material tracking forms are kept in a binder in the SRF EMF Manager's office. The MSDSs are added to the MSDS file kept in G75E2 ESB and to this Chemical Hygiene Plan, appendix B.

All containers must be brought to a SRF staff member to receive an approved chemical label, which is signed and dated by the SRF staff member. The chemical container must be appropriate for the chemical and it must be labeled appropriately with the full chemical name to receive an approved chemical label. Only containers with approved chemical labels may be taken into the lab.

A chemical must be approved before being brought or stored into the lab, even if the chemical is only going to be used one time.

SECTION 5.3.3: CHEMICAL HANDLING

All wet chemistry or any work with open chemical vessels must be performed in the fume hood. Users should be working with all chemicals at arm's length in the back half of the hood for their protection.

Users working in G75E2 are required wear lab coat and nitrile gloves. Lab coats protect from splashes and nitrile gloves are resistant to some chemicals. However, users are required to wear additional personal protective equipment (PPE) when working with specific chemicals.

When working in the fume hood:

- Check for appropriate chemical ratios. Improperly mixed chemicals may create dangerous reactions or dangerous fumes.
- Use the appropriate PPE and type of gloves for chemical usage
- Wear splash goggles when working with chemical processes
- Keep the work area inside the hood clean and free from obstructions.
- Always use appropriate vessels.
- Never heat solvents in closed beakers.
- Do not store items in the hood.
- Do not leave unlabeled chemicals unattended in a hood.
- Always properly label beakers.

Users working at the fume hood are required to wear the appropriate level of PPE determined for their process.

When working with Osmium Tetroxide, users must wear splash goggles, buttoned lab coat and double nitrile gloves, making sure there is no exposed skin between gloves and lab coat sleeves at any time.

First, remove the doubled container that has the Osmium Tetroxide from the refrigerator. Bring it to the fume hood. Do NOT open the containers under any circumstance while it is not under the fume hood. Once under the hood, the doubled container can be opened and the Osmium Tetroxide vial can be removed. Just keep the vial opened long enough to remove the amount of liquid you need for your process. Then, close the vial and put it back inside the containers. Close the doubled container and place it back in the refrigerator.

SECTION 5.3.4: ACCIDENTAL CHEMICAL EXPOSURE

Users are required to wear appropriate personal protective equipment (PPE) for their safety. If at any time a piece of PPE becomes damaged or torn, it should be replaced immediately. If a user has been exposed to a chemical, the following procedures should be followed immediately.

For eye exposure:

1. Remove all contaminated clothing and gloves.
2. Initiate water flow at eyewash station by either pressing on the pedal or the hand lever.
3. Hold eyes open with fingers and lower face into eyewash bowl, so that water is rinsing the eyes.
4. Keep eyes open and rotate.
5. Flush eyes for a total of 15 minutes.
6. Seek emergency medical attention.

For exposure to any part of the body:

WARNING: Do not wipe off the chemical; you will only increase area of contact! Increasing area of contact will increase absorption through the skin and may result in faster or more sever reaction or poisoning.

1. Remove all contaminated clothing and gloves.
2. Initiate water flow at safety show by pulling down ring.
3. Flush contaminated area for a total of 15 minutes.
4. Seek emergency medical attention

For exposure to Osmium Tetroxide:

1. Remove all contaminated clothing and gloves.
2. Flush contaminated area for a total of 15 minutes.
3. For eye exposure: Flush opened eyes with water for at least 15 minutes.
4. Inhalation exposure: if osmium tetroxide vapor has been inhaled from a spill, move the victim to fresh air immediately.
5. In all cases, seek emergency medical attention!

In case of MEDCIAL EMERGENCY contact:

9-911 from any campus phone
or
Campus Security
Phone: **(304)293-3136 (293-COPS)**

If going to the hospital for medical assistance, make sure you:

- Take a copy of the MSDS with you
- Inform medical personnel if you have been working with or been exposed to Osmium Tetroxide.

SECTION 5.3.5: CHEMICAL SPILL RESPONSE

If at any time a user is unsure of how to clean up a spill or is uncomfortable in trying to clean up the spill they should immediately evacuate the area and seek assistance. Users should attempt to only contain the spill and then seek assistance from the SRF staff. Personal protective equipment should be worn at all times.

If a chemical has been spilled in the lab G75E2:

A spill kit is kept in the room. Users should only attempt to contain the spill if it is less than one gallon.

To contain the spill:

1. Open the spill kit and follow its instructions.
2. Do not discard the spill kit.
3. Once the spill has been contained, seek SRF staff assistance.
4. The SRF staff will contact EH&S for pickup.

If Osmium Tetroxide has been spilled in the lab G75E2:

In the event of a spill, take appropriate actions to prevent exposure of osmium to everyone in the room, and to avoid the spread of contamination. If the spill is small and manageable (less than 2 ml):

1. Alert everyone in the immediate area to evacuate.
2. Isolate the area to prevent the spread of contamination.
3. Wear appropriate PPE (double nitrile gloves, buttoned lab coat and safety splash goggles)

4. Contact SRF staff personnel immediately.
5. SRF staff will cover the spill with inert absorbent (from spill kit or kitty litter) that has been infused with vegetable oil (corn oil preferred)
6. Scoop the contaminated material up and place it in a glass or plastic container (jar or pail) with a tight fitting lid.
7. This container will be put inside the solid waste container in the lab.
8. Wash the area of the spill with an aqueous solution of sodium sulfite.
9. Clean the area again with detergent solution.
10. Remove contaminated PPE carefully and place it in the solid waste container.
11. Label the waste container with a properly completed hazardous waste label and notify EH&S immediately for pickup.

At no time, will the BioSample Prep Lab have Osmium Tetroxide in amounts larger than 2 ml.

In case of an ACUTELY HAZARDOUS SPILL contact in the following order:

Environmental Health & Safety
Phone: **(304)293-3792**

Then Call,
Marcela Redigolo, Electron Microscopy Facility Manager
Cell Phone: **(304) 680-3007**
Office Phone: **(304) 293-9973**
Room: **G75D ESB**

or

Kolin Brown, SRF Chemical Hygiene Officer
Cell Phone: **(304)366-6551**
Office Phone: **(304)293-9683**
Room: **G75D ESB**
or

If no one responds to any numbers above, then contact:

Kenny Claudio
Cell Phone: **(304) 216-4858**
Office Phone: **(304) 293-4091**
Room**: 373A MRB**

Royce Watts,
Cell Phone: **(304) 288-6762**
Office Phone: **(304) 293-4124**
Room: **377A MRB**

SECTION 5.3.6: CHEMICAL DISPOSAL

All chemicals inside room G75E2 must be captured in waste jars.

To dispose of Osmium Tetroxide:

Osmium Tetroxide disposal should only be performed by the laboratory manager. To reduce hazards involved in discarding osmium tetroxide, the following neutralizing procedure will be used:

1. All these steps should be complete in the fumehood and wearing proper PPE.
2. A 2% solution of osmium tetroxide can be fully neutralized by twice its volume of vegetable oil (corn oil is preferred because of its high percentage of unsaturated bonds). For example, for every 10 ml of 2% osmium tetroxide solution, 20 ml of corn oil is required.
3. Pour the corn oil into the osmium tetroxide solution, and wait for the oil to completely turn black.
4. To test if the osmium tetroxide is fully neutralized, hold a piece of filter paper soaked in corn oil over the solution. Blackening indicates that osmium tetroxide is still present and more corn oil should be added.
5. Aqueous solutions contaminated with osmium tetroxide can be fully neutralized by adding sodium sulfide or sodium sulfite to reduce osmium tetroxide to less hazardous forms.
6. Dispose of neutralized solutions and all materials used for the procedures as hazardous waste.

All solid materials used to manipulate osmium tetroxide must be disposed in the solid waste container for osmium tetroxide. This includes gloves, tissues, manipulation tools such as wood sticks, wipes, plastic pipettes, etc. The solid container is clearly labeled and dated. This container will be inspected weekly by an SRF manager, and the facility manager will request EH&S pickup if the container has any waste.

To dispose of all other chemicals or chemical mixtures:
Used chemicals must be captured in chemical waste jars. All chemical waste is captured and stored in an appropriately chosen waste jar which is properly labeled with the word WASTE. The jar label should clearly list all contents of the waste jar. Ideally, the waste jar should be an empty original container of the same chemical being disposed of. Each chemical or mixture should have its own waste jar. The only waste jar that should be used for multiple chemicals is the alcohol waste jar; this jar may be used to dispose of methanol, isopropanol, and ethanol.

Waste jars should be closed when not in use and properly stored.

When a waste jar is full, it will be marked with a label stating "Hazardous Waste" and dated. SRF staff will contact EH&S to have it removed.

WARNING: Do not pour a hot liquid into a waste jar! Allow the hot chemical or chemical mixture to cool before adding it to a waste jar. Sealing a hot liquid in a waste jar may cause the jar to explode.

WARNING: Do not seal a waste jar if the material is highly reactive! If the waste jar heats up, or if it fumes, keep the jar open in the fume hood and contact a SRF staff member immediately for assistance. Sealing a chemical reaction in a waste jar may cause the jar to explode.

SECTION 5.4: SHARPS AND BROKEN GLASS DISPOSAL (PHYSICAL HAZARD)

A broken glass disposal box is kept in the lab, room G75E2. This box is for the disposing of broken glass, used glass knives from the ultramicrotome and Pasteur (glass) pipettes. SRF staff members inspect this box monthly. When full, the box is sealed and disposed with the laboratory trash.

Sharps disposal boxes are kept on top of the workbenches in the lab. The boxes are primarily for the disposal of razor blades, though any sharp may be disposed of here, including needles. SRF staff members inspect this box monthly. When full, the box is sealed and properly disposed.

WARNING: Do NOT recap used needles for disposal.

In the case of a sharp that is contaminated with osmium tetroxide, it should be discarded into a solid waste container designated for osmium tetroxide, not a sharps disposal box.A SRF staff member should be alerted immediately.

SECTION 5.5: LIQUID NITROGEN

Liquid nitrogen (LN_2) is inert, colorless, odorless, non-corrosive, non-flammable, tasteless, extremely cold, and has no warning properties. Special care must be taken by persons who handle or work in areas where liquid nitrogen is used. The hazards associated with LN_2 include:

- Over-pressurization and explosion due to LN_2 vaporizing to nitrogen gas (700x expansion ratio) in unvented containers.
- Severe burns caused by exposure to cold temperatures.
- Asphyxiation due to displacement of oxygen in the air in confined work areas.

Humans cannot reliably detect the presence of nitrogen. Liquid nitrogen has a 700x expansion ratio which may create physical hazards and injuries from the explosion of unvented containers. Extensive tissue damage or burns can result from exposure to LN_2 or cold nitrogen vapors.

Asphyxiation may result from the displacement of oxygen in the air with nitrogen to levels where there is insufficient oxygen to support life. Inhalation of oxygen deficient air can cause dizziness, nausea, vomiting, loss of consciousness, and death.

When working in the lab the body must be protected with pants and closed-toe shoes as required by the dress code for G75D entrance.The following personal protective equipment is required when handling or using LN_2:

- **Water proof thermal insulated gloves** (e.g., cryo-gloves):- Hands must be protected with water proof thermal insulated gloves that can be quickly removed if LN_2 is spilled on them. Insulated gloves are not intended for submersing hands into LN_2.

- **Cryo-aprons**:- Thermal insulated aprons are available and should be worn.

- **Safety goggles**:- Eyes are most sensitive to the extreme cold of LN_2 and its vapors. Over-pressurization may result in the explosion of improperly vented equipment. Chemical splash goggles must be worn when handling LN_2.

- **Full face shield**:- Face must be protected against splashes and spills of liquid nitrogen by a face shield.

People handing liquid nitrogen are required to wear appropriate personal protective equipment (PPE) for their safety. If at any time a piece of PPE becomes damaged or torn, it should be replaced immediately.

Although Liquid Nitrogen is not physically present in this lab, in case of certain specific samples, the protocol for preparation for electron microscopy study may require the use of a small quantity (less than 1L) of liquid nitrogen. A SRF staff member should be notified when liquid nitrogen is being brought into the lab. In these cases, users should follow all safety protocols to work with it.

SECTION 5.5.1: ACCIDENTAL EXPOSURE

Users are required to wear appropriate personal protective equipment (PPE) for their safety. If at any time a piece of PPE becomes damaged or torn, it should be replaced immediately. If a user has been exposed to liquid nitrogen, the following procedures should be followed immediately.

For frostbite exposure:

1. Remove all contaminated clothing and gloves, and any clothing item that may restrict circulation to the frozen area.
2. Do not rub frozen parts, as tissue damage may result.
3. Place the affected area in a warm water bath that has a temperature not exceeding 105°F (40°C).
4. Never use dry heat!!

In case of MEDCIAL EMERGENCY contact:

9-911 from any campus phone
or
Campus Security
Phone: **(304)293-3136 (293-COPS)**

If going to the hospital for medical assistance, make sure you:

- Take a copy of the MSDS with you
- Inform medical personnel you were exposed to liquid nitrogen.

SECTION 5.5.2: LIQUID NITROGEN SPILL RESPONSE

Personal protective equipment should be worn at all times. If a significant quantity of liquid nitrogen has escaped, or been spilt, the area affected will not contain adequate oxygen to support life. The area should be immediately evacuated and the following personnel should be contacted:

Environmental Health & Safety
Phone: **(304)293-3792**

Then call,

Marcela Redigolo, Electron Microscopy Facility Manager
Cell Phone: **(304) 680-3007**
Office Phone: **(304) 293-9973**
Room: **G75D ESB**

Or

Kolin Brown, Cleanroom Manager
Cell Phone: **(304)366-6551**

Office Phone: **(304)293-9683**
Room: **G75D ESB**

If no one responds to any numbers above, then contact:

Kenny Claudio
Cell Phone: **(304) 216-4858**
Office Phone: **(304) 293-4091**
Room**: 373A MRB**

Royce Watts,
Cell Phone: **(304) 288-6762**
Office Phone: **(304) 293-4124**
Room: **377A MRB**

If a person is showing symptoms of mild or severe asphyxia, they should be moved to an area with fresh air. If they are not conscious, security should be called immediately and resuscitation started by a qualified first aid officer or physician.

Cold burns from liquid nitrogen should be immediately and continually flushed with tepid or unheated tap water.

Professional medical advice should always be sought urgently for significant cold burns and asphyxia. Persons affected may need urgent medical treatment at a hospital.

SECTION 5.6: GAS SAFETY

The following gas cylinder is typically used by the CO2 Critical Point Dryer inside the biosample prep lab room, G75E2:

- Carbon Dioxide, bone dry.

Users should not have to open, close, or make adjustments with the regulators. Adjustments to regulators should be done only by SRF EMF staff.

The cylinder in use is mounted securely against a wall. All cylinders are transported using a cylinder hand truck. Eye protection or face shield is to be worn when changing cylinders. Users should communicate if a cylinder empties while running the critical point dryer. A new cylinder will be installed by a SRF staff member.

BNRF Lab – G75E ESB and EMF Lab – G75E2 ESB
WVU SRF Bio-Nano Research Facility and Electron Microscopy Facility

APPENDIX A

STANDARD OPERATING PROCEDURES

BNRF LAB – rooms G75E and G75E1 ESB
EMF LAB – room G75E2 ESB

APPENDIX B

CHEMICAL INVENTORIES AND MATERIAL SAFETY DATA SHEET (MSDS)

BNRF LAB – rooms G75E and G75E1 ESB
EMF LAB – room G75E2 ESB