

**Arizona State University
Standard Operating Procedures Manual**

Biosafety Level 1 Laboratory

of
Dr. David Nielsen

Associate Professor

SEMTE

BDC C406, C414, C496, C498 and GWC 627, 631

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Arizona State University
Biosafety Standard Operating Procedures
BDC C406, C414, C496, C498, GWC 627
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PURPOSE

This manual applies to all research staff, hosted visitors and guests, volunteers, building staff, and service staff who enter the laboratory. This manual will be reviewed annually by the Principal Investigator or Laboratory Supervisor for changes or corrections to ensure that it is accurate.

I. Introduction to General Safety and Training for the BSL 1 Laboratory

A. Required Training

The minimum requirements for qualification to work in the Nielsen BSL 1 lab are:

- 1) Current Environmental Health & Safety (EH&S) trainings including Biosafety, Lab Safety, Hazardous Waste, and Fire Safety and Prevention

Laboratory personnel shall demonstrate the following:

- Willingness to follow established laboratory safety guidelines and these standard operating procedures.

This document will provide the basis of training in conjunction with EH&S training.

Dr. Nielsen will provide information and arrange for training at the time of an individual's initial assignment to the lab. Dr. Nielsen will arrange for refresher training at least annually and when there are any changes in processes or procedures.

For more information concerning training, also see the ASU Biosafety Manual on the EH&S web site.

B. Administrative Procedures

It is the responsibility of each employee to carefully consider every action taken in the BSL1 lab and its potential impact on possible exposure or contamination, and to follow established Standard Operating Procedures (SOPs) and protocols diligently and without variance.

All employees will read and adhere to the ASU Biosafety Manual and to the SOP Manual for Dr. Nielsen's laboratory. All employees will use pertinent sections in the Biosafety in Microbiological and Biomedical Laboratories, CDC-NIH, 5th edition as a guideline and reference. Copies of these documents will be made available in the lab.

All employees will attend the Arizona State University EH&S courses in Laboratory Safety Training, Biological Safety Training, Respiratory Protection Training, and Fire Safety/Fire Prevention. Records of certification will be kept on file by Human Resources and EHS.

No employee will be trained to work in the lab without the express permission of Dr. Nielsen.

New SOPs and protocols must be approved by the PI before initiation.

Current SOPs and protocols will be reviewed and/or revised by Dr. Nielsen or Laboratory Manager every 12 months.

C. Description of Laboratory

The lab has been equipped with the following major pieces of equipment: an HPLC (Agilent 1200 series) equipped with DAD and RID detectors, a GC (Agilent 5890) with FID and TCD detectors, UV/Vis spectrophotometers (Beckman Coulter DU800, Thermo BioMate 3, GE NanoVue Plus), multiple microplate readers (Molecular Devices iD3, Thermo Multiskan Spectrum), two incubator shakers (New Brunswick Innova 44R (2)), five bench-scale bioreactors (Sartorius Biostat B+, New Brunswick BioFlo 110), a dual chamber static incubator (VWR 1565), two biological safety cabinets (Baker Co.), four PCR thermal cyclers (Bio-Rad, Applied Biosystems, Techne), electrophoresis equipment (Bio-Rad), and a large capacity centrifuge (Beckman Coulter Allegra X-22R) with multiple rotor configurations. We also maintain two walk-in Environmental Growth Chambers with controls for temperature, light intensity, and feed gas (flow rate and CO₂ content), as well as three additional standalone, reach-in units with temperature, humidity, and CO₂ atmosphere controls, including one that also controls light intensity (Caron Plant Growth Chamber). The lab has also been outfitted with a chromatography refrigerator, a -20°C freezer (Sanyo), two -80°C freezers (Revco, Thermo), a high purity water system (Millipore), multiple balances and microcentrifuges, mass flow controllers, pH analyzers, a rotary evaporator, a chemical fume hood, several pumps and other liquid handling equipment, a microwave, and other supporting peripherals consistent with such facilities. The lab is equipped with multiple PCs for data acquisition and processing. The lab has access to two large autoclaves (Steris) located on the same floor.

D. General Laboratory Safety

1. Laboratory employees must immediately notify the laboratory manager or PI in case of an accident, injury, illness, or overt exposure associated with laboratory activities.
2. No eating, drinking, smoking, handling contact lenses, or applying cosmetics in the lab at any time.

3. No animals or minors (persons under the age of 18), or immuno-compromised persons will be allowed to enter the lab at any time.
4. Food, medications, or cosmetics should not be brought into the lab for storage or later use. Food is stored outside in areas designated specifically for that purpose.
5. No open-toed shoes or sandals are allowed in the laboratory.
6. PPE includes gloves, lab coat, and eye protection.
7. All skin defects such as cuts, abrasions, ulcers, areas of dermatitis, etc. should be covered with an occlusive bandage.
8. Mouth pipetting is prohibited; mechanical pipetting devices are to be used at all times.
9. All procedures are to be performed carefully to minimize the creation of splashes or aerosols.
10. Follow all manufacturer's instructions and SOPs when using any of the laboratory equipment.
11. Wash hands:
 - after removing gloves, and
 - before leaving the laboratory.
12. Razor blades, scalpels, and hypodermic needles ("sharps") should be discarded into the "sharps" container in the biosafety cabinet. DO NOT recap needles. See Dr. Nielsen for training on proper handling of needles.
13. Work surfaces will be decontaminated as needed by spraying with 70% isopropanol solution and wiping clean with paper towel after 1 minute.

All cultures, stocks, and other regulated wastes are decontaminated by treatment with 10% bleach before contacting EH&S for pick-up and disposal.

NOTE: No biological agent-containing material should be allowed into any drain connected to the sanitary sewer system (e.g., from a sink) unless the method of inactivation has been pre-approved by the Department of Environmental Health and Safety. Please call 480.965.1823 to obtain pre-approval.

E. General Biosafety Cabinet (“Hood”) Safety

1. Turn on the blower in the cabinet at least 10 minutes before placing infectious materials into the hood.
2. Check the certification sticker and Magnehelic gauge to verify that the biosafety cabinet is working properly.
3. Check the air flow indicator to verify that the air flow is operating properly.
4. Lightly spray surfaces with 70% isopropanol solution and let air dry
5. Gloves must be worn at all times.
6. DO NOT disrupt the airflow through the hood by placing ANY item on the grills or by opening the door to the corridor.
7. DO NOT store items in the hood.
8. In general, the interior of the hood should be considered to be a contaminated zone, even though every effort is made to keep the surfaces clean, as is consistent with accepted good microbiological practice and sterile technique.
9. Clean the inside surfaces of the BSC with 70% isopropanol and let air dry after completion of work.
10. Allow the blower to run for at least 10 minutes following use.
11. The UV light is turned on between procedures (at least 5 minutes) and at the end of the day (all night). UV lights must be turned off before work begins in the hood. **Do not look directly at UV lights as this can cause eye damage.**

F. General Accident Procedures

Spills - Apply paper towels to absorb the spill, and then soak paper towels with 20% bleach solution. For spills outside the biosafety hood, alert others in the area.

Follow all aspects of the emergency SOPs without exception.

II. Standard Operating Procedures

A. 1000 – Containment Requirements

1001 - Laboratory

1001.1 Entering the lab to begin work

- A. Put on protective outerwear, including safety glasses, lab coat, and gloves.
- B. Gather all materials for the experiment.

1001.2 Exiting Laboratory

Before exiting the lab, be sure that all required documentation has been completed, the hood and work area are clean, all contaminated waste materials are disposed of properly, and stocks have been returned to the proper storage area. Wash your hands.

1002 - Specimen Transport

Transport of biological materials to another building or lab within the same building should be done in a covered container. If the samples are infectious, use a secondary container and label it with the contents and a contact person/phone number.

1003 - Work within Laboratories *BDC C406, C414, C496, C498, GWC 627*

During work with all microbiological materials, persons must be wearing safety glasses, lab coat, and gloves.

1003.1 Preparation of primary containers of agent stock solutions and manipulations of primary containers of agents should be conducted in a biological safety cabinet.

1003.2 The user should verify inward airflow of the biological safety cabinet before initiating work by checking the Magnehelic.

1003.3 All work should be done within the operationally effective zone of the biological safety cabinet.

1003.4 Discard pipets and tips appropriately in a hard-sided waste container lined with a red biohazardous waste bag.

1003.5 The interior of the hood should be cleaned periodically.

1003.6 When vacuum lines are used with systems containing agents, they will be protected with in-line filters to prevent entry of agents into the lines, and will be protected by a liquid trap containing bleach. **NOTE: No biological agent-containing material should be allowed into any drain connected to the sanitary sewer system (e.g., from a sink) unless the method of inactivation has been pre-approved by the Department of Environmental Health and Safety. Please call 480.965.1823 to obtain pre-approval.**

B. 2000 – Proper Use of Equipment

2001 - Biological Safety Cabinets

- 2001.1 – To assure sterility inside the cabinet and establish proper air flow for containment, the blower should be turned on at least ten minutes before infectious materials are to be put into the biosafety cabinet.
- 2001.2 – Biosafety cabinets must be certified prior to use. A qualified outside contractor must certify these cabinets annually. Check the certification sticker on the front of the unit to verify your biosafety cabinet's condition.
- 2001.3 – The biosafety cabinet air flow ("Magnehelic") gauge should be checked (reading is equal to approximately 0.5 inches) to assure proper operation of the cabinet before placing any materials into it. Readings indicate relative pressure drop across the HEPA filter. Higher readings may, therefore, indicate filter clogging. Zero readings may indicate loss of filter integrity. In either of these cases, notify the Laboratory Manager or PI.
- 2001.4 – NEVER place anything over the front or rear grill of a biosafety cabinet.
- 2001.5 – Disrupting the airflow into the front grill allows contaminated air from inside the cabinet to blow into the lab or directly at the person sitting at the cabinet. It also allows non-sterile air from the room to blow into the biosafety cabinet over the experiments.
- 2001.6 – Materials should be placed in the cabinet so as not to block air flow into the rear grill. Leave a few inches for air to flow around objects. Any disruption of the air flow in the cabinet decreases its effectiveness.
- 2001.7 – Before manipulating infectious materials, make sure that you have everything you need in the cabinet. The fewer times you pull your hands out of the cabinet, the less disruption of the air flow.
- 2001.8 – Work should be performed in the center of the work surface of the cabinet whenever possible. Work outward progressing from clean to dirty (contaminated). However, infectious agents should not be placed directly adjacent to or directly on the intake grills.
- 2001.9 – After manipulating infectious agents, make sure all containers are tightly closed.

2001.10 – All waste and disposable items generated by work in the cabinet should be discarded in the appropriate waste container after completing all work.

2001.11 – After the cabinet has been emptied, spray inner surfaces with 70% isopropanol and let air dry. Do not shut down the blower.

2001.12 – The bleach in the vacuum traps must be changed after one week of use or when the flask is half full. **NOTE: No biological agent-containing material should be allowed into any drain connected to the sanitary sewer system (e.g., from a sink) unless the method of inactivation has been pre-approved by the Department of Environmental Health and Safety. Please call 480.965.1823 to obtain pre-approval.**

NOTE: Please see additional SOP on Biological Safety Cabinets below, as well as the section on Biological Safety Cabinets in the ASU Biosafety Manual for more information.

NOTE: Though Class IIB cabinets are hard-ducted (so that all air is removed from the room), Class IIA cabinets recirculate about 70% of the air inside themselves and exhaust the remainder to the lab. Any use of volatile solvents, such as absolute ethanol, should be kept to a minimum or done elsewhere. **Dangerously high levels of volatile vapors can accumulate inside the cabinet and pose a threat of fire or explosion.**

2002 – Incubators

2002.1 – Upright Incubators

- A. Incubators are normally set at 30, 32, or 37° C.
- B. Temperature should be checked each day by all users.
- C. Operation manuals are located in the drawer labeled 'equipment manuals' in the lab and/or electronically on our lab website.
- D. Decontaminate incubators at least every 4 months.

2003 – Centrifuges

All centrifuges should be inspected before use for obstructions. All samples must be balanced prior to starting the centrifuge.

2004 – Autoclave

See additional SOP on autoclaves below.

2005 – Emergency Equipment

2005.1 – Fire Extinguisher, located beside the main door to the lab.

A. Operation

1. Fire extinguishers should be used only if the fire is small and confined to one small area! **USE JUDGEMENT IN THIS! DO NOT CREATE A LIFE-THREATENING SITUATION WHILE TRYING TO EXTINGUISH A FIRE!**
2. To operate, pull the pin to release the handle.
3. Stand at a safe distance from the fire.
4. Aim the nozzle at the base of the fire, squeeze the handle to discharge the agent, and sweep completely left and right until a few seconds after seeing no fire.

B. Maintenance

Fire extinguishers are inspected annually by EH&S. Check the gauge periodically to ensure operational status. Call EH&S at 965-1823 if you have any questions.

2006 – Repair and Service

Notify Dr. Nielsen and Fred Pena of any malfunctioning equipment at the earliest observation of the associated problem.

C. 3000 – Operational Procedures

3001 – Working outside the Biosafety Cabinet

Working outside the hood includes such actions as transporting samples from the hood to a centrifuge, incubator, sonicator, or water bath.

3001.1 – Vials or tubes being transported to the centrifuge, water bath, etc. should be in a stable rack.

3001.2 – **No liquid should be allowed into any drain connected to the sanitary sewer system (e.g., from a sink) unless the method of inactivation has been pre-approved by the Department of Environmental Health and Safety. Please call 480.965.1823 to obtain pre-approval.**

3002 – Maintenance of Laboratory

3002.1 Keep your personal workstation clean and organized.

3002.2 Return all reagents and chemicals to their prescribed shelf or cabinet.

D. 4000 – Experimental Procedures

See lab specific training manual located in lab and electronically on the lab website for specific details on experimental procedures.

E. 5000 – Safety Checks and Emergency Procedures

5001 – Training and Orientation

All employees will attend the Arizona State University EH&S courses in Laboratory Safety Training, Biological Safety Training, and Fire Safety/Fire Prevention (Respiratory Protection Training and Radiation Safety Training as needed), and annual refreshers.

5002 – Personal Protective Equipment (PPE)

5002.1 When using a biological safety cabinet, protective clothing, including gloves and a long-sleeved body covering (lab coat) should be worn so that hands and arms are completely covered to prevent contamination of cultures, skin and street clothing.

5002.2 Eye protection should be worn when handling infectious organisms or chemicals.

5002.3 These requirements also apply to anyone working in the area while someone else is working at the biosafety cabinet.

5003 – Waste Removal from the Lab

Place full biohazard waste bags in the gray bins located in BDC C428B for sterilization and EH&S pick-up.

5004 – Management of Spills

Apply paper towels to absorb biological spills, and then soak paper towels with 20% bleach solution. For spills outside the biosafety hood, alert others in the area.

Notify the PI. The PI will contact EH&S and Campus Health if there was an exposure.

5005 – Management of Accidental Exposures

In the event of an exposure to an infectious agent or material:

Intact skin

- Remove contaminated clothing.
- Vigorously wash contaminated skin for 1 minute with soap and water; there is a safety shower in the 6th floor hallway. Familiarize yourself with the location before beginning work in the lab.
- Inform Dr. Nielsen and seek medical attention at the ASU Campus Health Service.

Broken, cut, or damaged skin or puncture wound

- Remove contaminated clothing.
- Vigorously wash contaminated skin for 5 minutes with soap and water; there is a safety shower in the 6th floor hallway. Familiarize yourself with the location before beginning work in the lab.
- Inform Dr. Nielsen and seek medical attention at the ASU Campus Health Service, or call 911 for assistance.

Eye

- Immediately flush eyes for at least 15 minutes with water, using an eyewash, located at the sink on the west side of the lab (i.e., farthest from the door). Familiarize yourself with the location before beginning work in the lab.
- Hold eyelids away from your eyeball and rotate your eyes so that all surfaces may be washed thoroughly; and
- Inform Dr. Nielsen and seek medical attention at the ASU Campus Health Service, or call 911 for assistance.

Ingestion or Inhalation

- Inform Dr. Nielsen and seek medical attention at the ASU Campus Health Service, or call 911 for assistance.
- Do not induce vomiting unless advised to do so by a health care provider.

5006 – Medical Surveillance
Not applicable.

5007 – Emergency Phone Numbers and Procedures

5007.1 Emergency Phone Numbers

Fire and Medical Emergencies	911
Police	911
<i>David Nielsen (PI)</i> <i>Cell Phone:</i>	<i>617-233-3718</i>
	<i>Office Phone:</i>
<i>Fred Pena (Lab Manager)</i> <i>Office Phone:</i>	<i>480-965-4113</i>
	<i>480-965-4122</i>
Campus Health	480-965-3346
St. Luke’s Hospital	480-968-9411
ASU Police Department	480-965-3456
Department of Environmental Health & Safety (Main Number)	480-965-1823
Biosafety Officer (EHS)	480-965-5389

5007.2 Emergency Procedures

In responding to an emergency situation, all persons must be aware of these basic concerns:

Emergency Equipment:

- FIRE EXTINGUISHER** is located next to the main entrance.
- EYEWASH** is located next to the West sink.
- SAFETY SHOWER** is located adjacent to the East and West linear equipment rooms.

Order of Priority in an Emergency:

I. SAFETY FOR THE EMPLOYEE/STUDENT(S) IN THE LABORATORY.

Notify anyone else present in the lab.
 Safety for persons in the lab must be the first consideration. The circumstances of the emergency will determine the feasibility of carrying out site control.

II. DANGER OF OUTSIDE CONTAMINATION (Site Control):

Do not exit building wearing contaminated clothing or protective outerwear (gown, gloves).
 Leave biosafety cabinet on.
 Place covers on open containers of viable agents.

F. 6000 – Outside Operations

6001 – Receiving Reagents/Cells/Viruses

All cells and genetic materials must be sent to the Chemistry Stockroom. After pick-up, these should be transported back to the lab using an appropriate closed container.

6002 – Validation and History for SOP Manual (optional)

I hereby certify that I have reviewed the contents of this manual and that it reflects my current operating policy for the laboratories of Dr. Nielsen located in *BDC C406, C414, C496, C498, GWC 627* and associated spaces.

G. 7000 - Security

7001 – Security Protocols

All lab doors should be closed and locked at all times. Passwords to lab computers or web servers should not be written down or shared. Keys should be returned to the key rack when not in use.

III. Protocols

A. Emergency Spill Procedure: Biological

The following material are available in the laboratory to assist in the event of a biological spill: 20% bleach solution, paper towels, autoclavable bags, protective eyewear, latex gloves, and forceps (to pick up broken glass). The following individuals will be notified of any and all biological spill incidents: David Nielsen, Fred Pena. If the spill can be managed by laboratory personnel, proper personal protective equipment, including safety glasses, gloves, and a lab coat, will be worn to clean up the spill. The contaminated area will be cleaned using disinfectant allowing for a contact time of at least 20 minutes. Decontaminated waste material will be placed into a biological waste container lined with a biohazard bag. Clean-up equipment will be decontaminated using the following method: forceps will be washed with soap and water, followed by autoclaving. All personnel involved in the cleanup will wash their hands and any exposed body parts and/or clothing with soap and water. If the spill cannot be managed by laboratory personnel, EH&S (480-965-1823) will be notified for emergency assistance. In the event of a personal exposure: remove and place contaminated clothing articles in a biohazard bag, wash exposed skin with soap and water for 5 min; use safety shower for large exposures and eye wash as needed.

B. Biological Waste Handling Procedures

All biological waste in the research area will be handled and disposed of in accordance with federal, state, and local regulations as well as University policy. Materials containing recombinant DNA, synthetic nucleic acids, and genetically altered living organisms and their products are considered biohazardous waste. Any materials to be decontaminated outside of the research area will be transported in a durable, leak-proof, closed container.

All personnel who handle biological waste will successfully complete “Biosafety and Bloodborne Pathogens Training,” “ASU Autoclave Training Video,” and “Hazardous Waste Management Training” available at: <https://cfo.asu.edu/ehs-training>. For this research, biological waste will be treated using the following decontamination methods (select all that apply):

- Chemically decontaminated (e.g., bleach, quaternary ammonia compounds)
- Thermally inactivated (e.g., steam autoclave)
- Collected in biohazard containers for EHS pickup
- Other method (Contact EHS at 480-965-1823 to discuss further)

The specific procedures for biological waste management for this research are as follows:

Liquid biological waste: An appropriate chemically compatible disinfectant or a spore-tested steam autoclave will be used to decontaminate the waste (select all that apply).

An appropriate disinfectant (e.g., bleach, Lysol, Virex) will be added to the liquid biological waste to obtain the final concentration (e.g., 1:10, 1:25) and contact time per manufacturer’s guidelines. **List disinfectant, concentration, and contact time:** Bleach solution, 10% final conc., >1 h

A spore-tested steam autoclave will be used to disinfect the liquid biological waste (only if bleach or other hazardous chemicals are NOT present in the solution). **List autoclave location and cycle parameters:** BDC C428B, 40 min at 121°C

After decontamination, the liquid waste is considered chemical waste. Submit an online waste-pickup request at <https://cfo.asu.edu/waste-mgmt-and-shipping> or drain dispose if pre-approved by EHS. Contact Environmental Affairs at 480-965-1823 to request drain disposal approval. **List EHS approval date:**

Solid biological waste: A spore-tested steam autoclave will be used to decontaminate the waste or waste will be collected in biohazard drums and picked up by EHS (select all that apply).

Solid biological waste will be collected in an autoclavable biohazard bag (or heat-safe container) and decontaminated using an appropriate cycle in a spore-tested steam autoclave (only if bleach or other hazardous chemicals are NOT present in the materials to be autoclaved). After autoclaving, the waste will be placed in a red biohazard drum. Submit an online waste-pickup request when the red drum is two-thirds full at <https://cfo.asu.edu/waste-mgmt-and-shipping>. **List autoclave location and cycle parameters:** BDC C428B

No autoclave is available. The solid biological waste will be collected in double-lined, red biohazard bags and subsequently placed into a red biohazard drum. Submit an online waste-pickup request when the red drum is two-thirds full at <https://cfo.asu.edu/waste-mgmt-and-shipping>.

Solid human and animal waste may require special handling procedures. Please contact EHS Environmental Affairs at 480-965-1823 to discuss further.

All spills, injuries, equipment failures, or waste handling issues will be reported to EHS at 480-965-1823 and the individuals listed below.

List names and contact numbers:

David Nielsen, 480-965-4113

Fred Pena, 480-965-4122

For guidance on proper autoclave use and procedures for spore testing consult the ASU Autoclave Manual at <https://www.asu.edu/ehs/documents/asu-autoclave-safety-manual.pdf>

The EHS Biosafety/Biosecurity and Environmental Affairs teams are available to discuss biological waste handling procedures at 480-965-1823.

C. Safety Manuals

1. ASU Biosafety Manual
2. Biosafety in Microbiological and Biomedical Laboratories, CDC-NIH, 5th edition.

Dr. David Nielsen
Associate Professor
Chemical Engineering, SEMTE

Signature _____ Annual Review Date _____

History of Manual's Creation

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Author: David Nielsen

Reference Source(s): ASU EH&S, Biosafety and Biosecurity

APPENDICES

- A. Material Safety Data Sheets are maintained in a notebook located next to the main entrance of the laboratory.
- B. SOP: Safe Autoclave Operations
- C. SOP: Biological Safety Cabinets
- D. SOP: Biohazardous Sharps Handling and Disposal
- E. SOP: Biohazardous Liquid Spill Clean-up
- F. SOP: Biohazardous Waste Disposal
- G. SOP: Emergency Clean-up of Chemical Spills
- H. Needle Stick Incident Protocol
- I. SOP: Benzene
- J. SOP: Sodium azide
- K. Thiols, Mercaptans, and Other Stench Chemicals



STANDARD OPERATING PROCEDURES

Appendices

Nielsen Lab Chemical Engineering, SEMTE

**Covered Labs:
BDC C496, C414, C406, C498
GWC 627, 631**

**PI: David Nielsen
(480) 965-4113
David.R.Nielsen@asu.edu**

Last Modification Date: January 2022

STANDARD OPERATING PROCEDURES Safe Autoclave Operations

The purpose of this document is to provide standard operating procedures for the safe use of autoclaves. Autoclaving is a process used to destroy microorganisms and decontaminate biohazardous waste and microbiological equipment used at Biosafety Level 1, 2, 3 and 4.

1. Steris AMSCO 250LS , 2. Steris AMSCO SV-1262

Autoclave Make/Model: _____

Person Responsible and Phone #: **biodfacilities@asu.edu**

Located in Building/Room: **1. GWC 627, 2. BDC C428B**

HAZARDS

Autoclaves use high pressure and high temperature steam for sterilization. The potential safety risks for the operators include:

- Heat burns from hot materials and autoclave chamber walls and door.
- Steam burns from residual steam coming out from autoclave and materials on completion of cycle.
- Hot fluid scalds from boiling liquids and spillage in autoclave.
- Hand and arm injuries when closing the door.
- Body injury if there is an explosion.

SAFETY

To insure the health and safety of personnel using the autoclave, it is important for each department to maintain autoclaves and to train personnel in their proper use.

- The name of the person responsible for the autoclave shall be posted near the autoclave. This SOP should be posted on the outside to the autoclave.
- It is the supervisor's responsibility to ensure employees are trained before operating any autoclave unit.
- Procedural and instructional documents provided by the manufacturer must be followed.
- Personal protective clothing and equipment must be worn when loading and unloading the autoclave.
- Autoclaves must be inspected at least annually. Inspection services may be managed by your manufacturer's preventative maintenance contract. A basic visual inspection should be performed monthly by the person responsible for the autoclave. The inspection, service and repair records should be available upon request.
- Spore strips may be used to validate autoclave effectiveness.

PERSONAL PROTECTIVE EQUIPMENT

Equipment to protect against scalds and burns include:

- Heat-insulating gloves that provide complete coverage of hands and forearm
- Lab coat
- Eye protection
- Closed-toe footwear

OPERATOR INSTRUCTIONS

Training

All personnel who use autoclaves must have successfully completed a training session from their supervisor on the safe operating procedures. This requirement applies to both new and experienced personnel.

Material Preparation

Ensure that the material is safe for autoclaving:

- Samples containing solvents or substances that may emit toxic fumes should not be autoclaved.
- Do not autoclave bleach!

Glassware must be inspected for cracks prior to autoclaving.

Prepare and package material suitably:

- Loose dry materials must be wrapped or bagged in steam-penetrating paper or loosely covered with aluminum foil. Wrapping too tightly will impede steam penetration, decreasing effectiveness of the process.
- Loosen all lids to prevent pressure buildup. All containers must be covered by a loosened lid or steam-penetrating bung.
- Containers of liquid must not exceed two-thirds (2/3) full, with lids loosened.
- Glassware must be heat-resistant borosilicate.
- Plastics must be heat-resistant, i.e., polycarbonate (PC), PTFE ("Teflon") and most polypropylene (PP) items.
- Discarded sharps must be in a designated 'Sharps' container.
- All items must be tagged with autoclave tape.

Place items in secondary containers to secure and contain spills:

- Items should be placed in a stainless steel pan or other autoclavable container for their stability and ease of handling.
- Place containers of liquid, bags of agar plates, or other materials that may boil over or leak, into a secondary pan in the autoclave.
- The pan must be large enough to contain a total spill of the contents.
- Bags must not be tightly sealed as steam cannot penetrate.

Biohazardous waste must be processed according to ASU guidelines.

Loading Autoclave

- Wear lab coat, eye protection, heat-insulating gloves, and closed-toe shoes.
- Place material in autoclave. Do not mix incompatible materials.
- Do not overload; leave sufficient room for steam circulation. If necessary, place the container on its side to maximize steam penetration and avoid entrapment of air.
- Close and latch door firmly.

Operating Autoclave

- Close and lock door.
- Choose appropriate cycle (e.g., gravity, liquid, or dry cycle) for the material. Consult the autoclave manual for assistance in choosing a cycle. The manuals for operation of the autoclave should be located near the autoclave.
- Set appropriate time and temperature if you are using a customized cycle.
- Start your cycle and fill out the autoclave user log with your contact information. A completed cycle usually takes between 1-1.5 hours, depending on type of cycle.
- Do not attempt to open the door while autoclave is operating.
- If problems with your autoclave are perceived, abort cycle and report it to your PI immediately.

Unloading Autoclave

- Wear heat-insulating gloves, eye protection, lab coat, and closed-toe shoes.
- Ensure that the cycle has completed and both temperature and pressure have returned to a safe range.
- Wearing Personal Protective Equipment (PPE), stand back from the door as a precaution and carefully open door no more than 1 inch. This will release residual steam and allow pressure within liquids and containers to normalize.
- Allow the autoclaved load to stand for 10 minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to operator.
- Do not agitate containers of super-heated liquids or remove caps before unloading.
- Wearing heat-insulated gloves remove items from the autoclave and place them in an area which clearly indicates the items are 'hot' until the items cool to room temperature.
- Shut autoclave door.

Autoclave Use Log

- Entries must be placed in the log book each time the autoclave is used. These records are used for maintenance/service schedules and reporting of incidents, accidents and/or faults.
- Entries should include: operator's name, phone number, date, time and duration.
- The log book must be kept adjacent to the autoclave.
- An Autoclave Use Log example is provided in this document.

Maintenance and Repair

- No person shall operate the autoclave unless the autoclave is in good repair.
- Only qualified professionals are permitted to make repairs.
- Report possible malfunctions to
- Repairs are performed by your service contract or any other contractor you choose to hire.

CONTINGENCY PLANS

Equipment Malfunction

- If the autoclave does not operate exactly as expected, do not attempt to fix the problem. A notice shall be placed on the autoclave indicating that it is not to be used until the problem is diagnosed and corrected.
- Record the problem in the autoclave log book.
- Contact biodfacilities@asu.edu or your supervisor to report the problem.
- Only qualified professionals are permitted to make repairs.

Incident Response

- All incidents, including a spill or release of biohazardous materials and recombinant or synthetic nucleic acid molecules, must be reported to your supervisor and Biosafety & Biosecurity.
- If any injury occurs seek first aid or, if necessary, seek medical assistance at ASU Health Services or by dialing 911.
- If clothing is soaked in hot water/steam, remove clothing and place the injury in cool water.
- Place a notice on the autoclave indicating that it is not to be used until the cause of the incident is determined, procedures enacted to prevent future incidents, and the autoclave is deemed safe for operation.

Spill Clean-up

- Spills may occur from a boil-over or breakage of containers.
- No operation of the autoclave is allowed until the spill is cleaned up.
- The operator is responsible for clean-up of spills. Contain the spilled material using paper towels. Use your laboratory's spill kit if necessary. Wait until the autoclave and materials have cooled to room temperature before attempting clean-up.
- Review the Safety Data Sheet if appropriate, to determine appropriate PPE, spill cleanup and disposal protocols that are necessary.
- Dispose of the waste following the protocol appropriate for the material (e.g., red biohazard bag). If materials have been intermingled, follow the clean-up and disposal protocol for the most hazardous component of the mixture.
- Cracked glassware must be disposed of properly.
- Record the spill and clean-up procedure in the autoclave log book.

ASU Autoclave Training Video

The EH&S Biosafety/Biosecurity team is pleased to introduce the **ASU Autoclave Training Video**. The video provides instructions on how to properly load and unload an autoclave, the requisite personal protective equipment to be worn, the waste disposal procedures to be followed, and other important autoclave safety topics. For your convenience, this training video is now available on Blackboard in five separate modules:

Module 1: Overview of Autoclaves

Module 2: Preparing for Autoclave Use

Module 3: Loading the Autoclave

Module 4: Operating the Autoclave

Module 5: Unloading the Autoclave

This training video is intended to serve as supplemental instruction to hands-on training and is recommended for all individuals using autoclaves at ASU. Please be aware that individuals must receive hands-on training for the autoclaves assigned to their laboratory before using them. Consult with your laboratory manager for this hands-on training.

To self-enroll in the Blackboard training, please click on either of the following links. To receive credit for completing this training, please watch the video modules and take the corresponding test in Blackboard. A score of 80% or higher is required to pass the test.

EH&S Training Determination Link: <https://cfo.asu.edu/ehs-training>

Self-Enrollment Link: <http://www.asu.edu/go/blackboard/selfenroll/?cid=320120>

If proof-of-training is not required for your laboratory, the ASU Autoclave Training Video is also available on YouTube:

https://www.youtube.com/watch?v=rM_JTgLSKXk

STANDARD OPERATING PROCEDURES

Biological Safety Cabinets

The purpose of this guideline is to detail the safe operation of biological safety cabinets (BSC) in ASU laboratories and to ensure adequate containment of biological materials. The most common type of BSC at ASU is a Class II. Class II BSCs provide the microbe-free work environment necessary for cell culture propagation and handling of infectious organisms.

Biological Safety Cabinet Make/Model: Esco EQU/04-EBC-2A , Baker SterilGARD II

Person Responsible and Phone #: David Nielsen 5-4113

Located in Building/Room: BDC C498B, C414C

DEFINITIONS

Biological Safety Cabinet: A piece of equipment designed to protect the operator, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating substances containing infectious agents, such as viruses, bacteria and primary tissue cultures.

High Efficiency Particulate Air (HEPA) Filter: Filter that traps 99.97% of particles of 0.3 μm in diameter and 99.99% of particles of greater or smaller size, thus capturing all infectious agents and ensuring only microbe-free air is exhausted from the cabinet or directed to the work surface.

RESPONSIBILITIES

Principal Investigator

The Principal Investigator (PI) is responsible for ensuring personnel under their supervision are trained on the safe and proper use of the BSC and that correct procedures are followed.

Designated Person

The PI may designate a member of the lab to oversee the correct operation and maintenance of the BSC. This person shall:

- Periodically inspect the BSC to ensure its operational performance.
- Coordinate yearly BSC certification by the certified vendor. The dates of the most recent certification must be posted on the front of the BSC.
- Make any necessary arrangements for repairs and annual certification of the BSC.
- Report to PI unsafe practices by BSC users.
- Label the BSC when it is not operating correctly.

BSC Users

- BSC users must receive proper training from their PI on the safe and proper use of the BSC prior to use.
- BSC users must follow correct procedures.
- BSC users shall report any injuries, accidents or spills to their PI.
- BSCs users shall report any defects or malfunctions of the BSC to their PI or the designated person.

Procedures

Prior to Operation

- Read the Safety Data Sheets (SDS) for materials being used in a BSC. Note any precautions regarding the use of the chemical or microorganisms in the BSC.
- Lift the sash to the recommended height.
- Turn on the BSC fan 10 minutes before beginning work to allow adequate air filtration.
- Check the air intake and exhaust grilles for obstructions, and check the pressure gauge reading indicating HEPA filter load. If you do not know the acceptable reading for the cabinet, ask the certifier or consult your manufacturer's manual.
- If the cabinet is equipped with an alarm, test the alarm and switch it to the "ON" position.
- Confirm inward airflow by holding a piece of tissue at the middle of the edge of the viewing panel and ensuring it is drawn in.
- Decontaminate the cabinet surface and surface-decontaminate all materials to be placed inside the BSC (refer to the Cleaning and Disinfection section).
- Bulky items, such as waste containers and suction collection flasks, should be placed to one side of the interior of the cabinet.
- Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may impede proper airflow and the level of protection provided. Consider using wire racks or shelving to increase airflow around front and rear grills.
- Wear eye protection, a lab coat, closed-toe shoes and disposable gloves to protect yourself as well as your samples from contamination.
- Respirators may be required for some procedures. The nature of the Personal Protective Equipment (PPE) worn may vary according to the findings obtained from the biological risk assessment.

Operation

- Active work should flow from clean to contaminated areas across the work surface.
- Arms should be moved in and out slowly, perpendicular to the front opening to minimize disruption of the air curtain and laminar flow.
- Work as far to the back as possible, but within comfortable reach.
- Always use mechanical pipetting aids. Mouth pipetting is not allowed.
- Heat sources such as Bunsen burners are strictly prohibited inside the BSCs as they significantly disrupt the laminar flow of air.
- To sterilize bacteriological loops, micro-burners or electric "furnaces" may be used.
- Do not work in a BSC while a warning light or alarm is signaling.
- Locate liquid waste traps inside cabinet and use an in-line HEPA filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container (such as a durable plastic tray or box) to prevent breakage.

Cleaning and Disinfection

- When work is completed, all equipment and supplies from the BSC should be decontaminated and removed from the cabinet.
- The interior surfaces should also be wiped with an appropriate disinfectant that would kill any microorganisms that could be found in the cabinet. Corrosive chemicals such as bleach should be avoided, but if used, should be followed with a wipe down of sterile water or 70% ethanol.
- Allow the cabinet to run for 10 minutes.

Ultraviolet (UV) Lights

- Ultraviolet (UV) lamps are not recommended in BSCs nor are they necessary.
- If installed, UV lamps must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light.
- The lamps should be checked weekly with a UV meter to ensure that the appropriate intensity of UV light is being emitted.
- UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer.
- If the cabinet has a sliding sash, close the sash when operating the UV lamp.

Spills

In the event of a spill within a BSC, follow these steps:

1. Alert the other laboratory employees.
2. Leave the cabinet turned on.
3. While wearing gloves, spray or wipe cabinet walls, work surfaces and equipment with disinfectant equivalent to 1:10 bleach solution. If necessary, flood the work surface, as well as drain-pans and catch basins below the work surface, with disinfectant for a contact time of at least 20 minutes.
4. Report the spill to the laboratory's PI, who will report the spill to the Responsible Official if a select agent or toxin is involved.
5. Soak up disinfectant and spill with paper towels. Drain catch basin into a container. Lift front exhaust grill and tray and wipe all surfaces. Ensure that no paper towels or solid debris are blown into the area beneath the grill.
6. Autoclave all clean-up materials before disposal in the biohazard waste container.
7. Wash hands and any exposed surfaces thoroughly after the clean-up procedure.

Other Considerations

- All repairs to the BSC must be performed by a qualified technician. Any malfunction of the BSC must be reported and repaired before the BSC is used again.
- The BSC should be positioned in an isolated corner to minimize disruption of the air intake arising from traffic around the biosafety cabinet or drafts from doors, windows and/or air conditioning.
- The BSC must be certified by an NSF/ANSI Standard 49 qualified technician.
- The BSC must be decontaminated before filter changes and before being moved. The most common decontamination method is by fumigation with formaldehyde gas. BSC decontamination may only be performed by a certified contractor.
- Contact Biosafety & Biosecurity if you have any questions.

STANDARD OPERATING PROCEDURES

Biohazardous Sharps Handling and Disposal

All sharps must be handled and disposed in a manner that protects you and others from exposure and possible injury. This procedure applies to the handling and disposal of sharps and must be followed to:

1. Prevent personal injury
2. Prevent contamination of personnel or the environment.
3. Ensure proper containment of laboratory and infectious waste during collection, transfer, and disposal.

Person Responsible and Phone #: David Nielsen 5-4113

For labs in Building/Room: ~~GWC 603~~ BDC C406, C414, C496, C498, GWC 627, 631

DEFINITIONS

Sharps are items that can easily puncture the skin. Examples include needles, razor blades, and broken glass. All sharps must be handled and disposed in a manner that protects you and others from exposure and possible injury.

I. PURPOSE

1. This procedure outlines methods for safe handling and disposal of all discarded syringes, needles, or sharps used by research staff and students.

II. RESPONSIBILITY

1. It is the responsibility of all program staff to contribute to and comply with these procedures.
2. It is the responsibility of the Principal Investigator to provide and maintain a sufficient number of properly labeled sharps containers in procedural areas throughout the lab and as described in this procedure.

III. PROCEDURES

All needles, syringes, scalpel blades, disposable instrument, etc. will be disposed of at the point of use in rigid, leak proof sharps containers, labeled as biohazardous waste with the biohazardous symbol and phrase.

Always wear gloves and other appropriate personal protective equipment when performing procedures using needles and syringes.

Disposable syringe-needle units should be used for the injection or aspiration of infectious/hazardous materials.

Upon completion of a procedure involving use of a syringe and needle, discard entire needle and syringe system into a clearly labeled puncture – resistant hazardous waste container located in the work area.

Needles should never be recapped, bent, broken, removed, or otherwise manipulated by hand.

When a Sharps container is three-fourth's full, don gloves, seal container, and place in the designated area for collection and disposal.

Syringe disposal containers will be checked and changed on an as needed basis.

If a needle stick should occur, wash area with soap and water, report incident to your supervisor, and seek medical attention as soon as possible. ALL NEEDLE STICKS WILL BE REPORTED AT ONCE TO YOUR SUPERVISOR.

General Precautions

Substitute glassware for plastic-ware whenever possible. Routinely inspect glassware and remove from service items that are damaged, starred, cracked, or chipped.

Make sure lighting is adequate and the work space is not crowded for the task at hand.

Be alert at all times when handling sharps. Don't look away or become otherwise distracted while handling a sharp object.

Select rounded or blunt end devices when practicable.

Do not handle sharp objects (i.e., broken glass) with bare hands. Use mechanical devices.

Do not leave unprotected sharps (i.e., razor blades, scalpel tips, etc.) on bench tops or loose in drawers. Use protective shields, cases, Styrofoam blocks, tube holders, etc.

Use needle syringes only when absolutely necessary. If a needle syringe is absolutely necessary to the procedure.

Do not try to recap the needle of a syringe. An accidental puncture might occur. If absolutely necessary, use the one-handed technique.

Keep a sharps disposal container immediately accessible. Read the authorized sharps container manufacturer's instructions and recommended user training information prior to use. Do not allow it to get overfilled. Do not try to retrieve items from sharps disposal containers.

Broken glass must be discarded into a plastic-lined container with the label "Broken Glass", unless it is contaminated with biological materials. Broken glass contaminated with biological materials must be discarded in a red sharps disposal container.

Precautions for the Disposal of Sharps

Ensure that appropriate sharps disposal containers are available in the immediate work area: The sharps disposal container must be labeled with a biohazard symbol. Sharps disposal containers must be rigid, leak and puncture proof, and sealable.

Needles and needle-syringe units, whether infectious or not, must be placed in the sharps disposal container.

Sharps disposal containers storing needles and syringes contaminated with biological materials cannot be placed in the normal trash.

Sharps disposal container must not be overfilled. This hazard should be reported to the lab manager or PI immediately upon notice. If the sharps container is overfilled, obtain a new container and use forceps or tongs to remove protruding devices and place them in the new container. Make sure the sharps container being used is large enough to accommodate the entire device. Notify EH&S for assistance in removing the hazard, if necessary.

Do not try to retrieve items from sharps disposal containers.

Never force materials into a sharps container.

Safety hazards identified with the sharps disposal container, i.e. needles protruding from the container, needles not freely falling into the container, must be immediately reported to the lab manager or PI.

Disposal of Sharps Used with Pathological Agents

Ensure that appropriate sharps containers are available in the immediate work area:

- Must be labeled as "Sharps."
- Must be labeled with a biohazard symbol.
- Containers must be rigid, leak and puncture proof, and sealable. A commercially available plastic container that cannot be easily opened after sealing must be used for sharps that are contaminated with potentially infectious materials.

Contaminated sharps cannot be placed in the normal trash until effectively decontaminated, usually by steam autoclaving. Use autoclave indicator tape to demonstrate decontamination.

You may mix non-infectious sharps with potentially infectious sharps only if the entire contents of the container is treated as infectious and it is decontaminated before disposal, as described above.

Disposal of Sharps that Have Not Been Used with Pathological Agents

Appropriate containers must be available in the immediate work area. Containers must be rigid, leak and puncture proof, sealable, and labeled as "Sharps."

STANDARD OPERATING PROCEDURES

Biohazardous Liquid Spill Clean-up

The purpose of this guideline is to detail procedures to be utilized as a basis for cleaning spills of biological materials including potentially infectious agents, and/or recombinant or synthetic nucleic acid molecules, including 1) Minor Biological Spills and 2) Major Biological Spills.

Person Responsible and Phone #: David Nielsen 5-4113

For labs in Building/Room: ~~GWC 603~~ BDC C406, C414, C496, C498, GWC 627, 631

DEFINITIONS

Minor Biological Spill: one that the laboratory staff is capable of handling safely without the assistance of EH&S or external emergency personnel. Minor biological spills involve low risk materials (e.g. BSL-1) or spills of a small volume of material inside a biological safety cabinet or other primary containment device.

Major Biological Spill: one that requires assistance of EH&S and/or external emergency personnel. Major Biological Spills involve releases of BSL-2 or higher materials outside of a biological safety cabinet, or spills of such materials that involve excessive splashing or aerosol formation.

I. PURPOSE

1. This procedure outlines methods for safe handling and clean-up of biohazardous liquid spills throughout the lab. As the nature of biohazardous waste spills associated with our research activities will primarily constitute live liquid cultures containing bacteria and yeast and will be of sufficiently small volume (typically < 100 mL and always <3 L) the below information has been tailored to this end.

II. RESPONSIBILITY

1. It is the responsibility of all program staff to contribute to and comply with these procedures.
2. It is the responsibility of the facility manager to provide and maintain adequate materials needed for the decontamination and clean-up of biohazardous liquid spills.

III. PROCEDURES

1) Minor Biological Spills

If the spill occurred inside a biological safety cabinet, close the sash and allow the cabinet to operate for at least 15 minutes before proceeding with the spill cleanup.

Alert people in area of spill and secure the affected area.

If any material has been splashed on you, immediately wash the exposed area with soap and water. If the exposed area is the eye, flush with water only for 15 minutes. NOTE: It is not advisable to apply alcohol, bleach or other disinfectants to the exposed area.

Remove and disinfect any contaminated clothing.

If you have not been exposed, don the appropriate personal protective equipment for the cleanup operation, which include at a minimum liquid barrier gloves, a combination of safety glasses or full face shield and surgical mask, and lab coat.

Cover the spill with paper towels or other absorbent material to prevent further aerosol formation.

Pour an approved disinfectant gently over the covered spill, working from the outside inwards.

- For microbial and yeast cultures, use a solution of 10% bleach in water.

Wait at least 15 minutes for the disinfectant to penetrate through the contained spill and achieve the required contact time for disinfection.

Do not utilize hands if glass or other sharps are involved in the spill. Use a tool (e.g. shovel or forceps) to remove the absorbent material and debris. Place all materials in a biohazard bag for autoclaving and subsequent disposal.

Clean the original spill area with detergent.

Notify your supervisor of the incident.

2) Major Biological Spill

Alert personnel in the laboratory of the spill, and eliminate entrance of additional personnel via notification or posting of area.

If any material has been splashed on you, immediately wash the exposed area with soap and water. If the exposed area is the eye, flush with water only for 15 minutes. NOTE: It is not advisable to apply alcohol, bleach or other disinfectants to the exposed area.

Remove and disinfect any contaminated clothing.

Notify your supervisor and ASU Environmental Health and Safety (EH&S).

If the situation involves an imminently life-threatening injury call 911.

Have persons knowledgeable of incident and laboratory available to assist notifying additional EH&S and/or emergency personnel.

STANDARD OPERATING PROCEDURES

Biohazardous Waste Disposal

The purpose of this guideline is to detail procedures for the proper disposal of biohazardous wastes, including: 1) liquid cultures, 2) solid cultures, and 3) contaminated consumables.

Person Responsible and Phone #: David Nielsen 5-4113

For labs in Building/Room: ~~GWC 603~~ BDC C406, C414, C496, C498, GWC 627, 631

DEFINITIONS

Biohazardous waste includes any materials containing or that have been in contact with microorganisms, materials derived from microorganisms (e.g., recombinant DNA, RNA, proteins), and/or synthetically-derived nucleic acids or nucleotides, as well as any waste material that is potentially infectious to humans, plants, or animals.

I. PURPOSE

1. This procedure outlines methods for safe handling and disposal of all biohazardous waste materials generated by research staff in the lab.

II. RESPONSIBILITY

1. It is the responsibility of all program staff to contribute to and comply with these procedures.
2. It is the responsibility of the facility manager to provide and maintain adequate and sufficient materials for the safe handling, decontamination and disposal of biohazardous wastes throughout the lab and as described in this procedure.

III. PROCEDURES

All biological waste in the laboratory will be handled and disposed of in accordance with federal, state, and local regulations as well as University policy. Waste will be chemically disinfected (e.g., bleach, quaternary ammonia compounds), thermally inactivated (e.g., steam autoclave), or collected in biohazard containers for EH&S pickup. Any materials to be decontaminated outside the laboratory will be transported in a durable, leak-proof, closed container. All personnel who handle biological waste will successfully complete “Biosafety and Bloodborne Pathogens Training” and “Hazardous Waste Management Training” through EH&S. Enrollment procedures for these trainings are available here: <https://cfo.asu.edu/ehs-training>.

For the research in our laboratory, we will have liquid and solid biological waste. Procedures for waste management in our laboratory are provided below:

For liquid biological waste, an appropriate, chemically compatible disinfectant, or steam autoclave will be used to decontaminate the waste. An approved disinfectant (e.g., 10% bleach/water solution - 1 part concentrated bleach to 9 parts water) will be added to the liquid waste to obtain the appropriate final concentration and allowed to sit for at least 30 minutes. Alternatively, a steam autoclave can be used to disinfect the liquid biological waste only if no bleach is present in the solution. If approved for drain disposal, the chemically disinfected solution will be drain disposed and a logbook with the following information will be maintained in the laboratory: A) Date of Drain Disposal; B) Type of Waste; C) Volume of Total Waste Disposed; D) Method of Disinfection; E) pH of Final Solution. If the pH is <5 or >10 then drain disposal is not permitted and the waste will be collected in closable, leakproof containers labeled with a biohazard symbol for EH&S to pick-up.

For solid biological waste, a steam autoclave will be used. Waste will be collected in a heat-safe autoclave bag (or heat-safe container) and decontaminated using an appropriate cycle in the autoclave. Please consult <http://www.asu.edu/uagc/EHS/documents/autoclave-sop.pdf> for additional guidance and procedures for proper autoclave use. The autoclaved waste will be collected in a red biohazard bag and subsequently placed into a red biohazard drum. Prior to having the red drum two-thirds full, laboratory personnel will submit an online waste-pickup request at <https://cfo.asu.edu/ehs-environmentalaffairs>.

If waste is not appropriate for chemical disinfection or steam autoclave, it will be collected in double-lined, red biohazard bags and subsequently placed into a red biohazard drum. Prior to having the red drum two-thirds full, laboratory personnel will submit an online waste-pickup request at <https://cfo.asu.edu/ehs-environmentalaffairs>.

If animal waste is generated in the laboratory, it will be thermally disinfected in a steam autoclave, placed into a red-biohazard bag, and then placed into a yellow drum. If autoclaving is not suggested for the waste, it will be collected in double-lined, red biohazard bags and then placed into a yellow drum. Prior to having the yellow drum two-thirds full, laboratory personnel will submit an online waste-pickup request at <https://cfo.asu.edu/ehs-environmentalaffairs>.

Any spills, injuries, equipment failures, or waste handling problems will be reported to the PI at (480) 965-4113 and EH&S at (480) 965-1823.

The EH&S Biosafety and Environmental Affairs groups will be contacted regarding any final waste determinations not addressed by these procedures.

Note: Genetically altered living organisms and their products, and materials containing recombinant DNA or synthetic nucleic acids are also considered to be biohazardous waste and will be handled according to the procedures for solid or liquid biohazardous waste.

All requests for waste pick up can be made through ASU's [EHS Assistant](#) portal.

STANDARD OPERATING PROCEDURES

Emergency Clean-up of Chemical Spills

The purpose of this guideline is to detail steps to be taken (1) to prevent the spill of a hazardous chemical, and (2) to respond to a chemical spill that has occurred. (3) Information about spill kits is also given.

Person Responsible and Phone #: David Nielsen 5-4113

For labs in Building/Room: ~~GWC 603~~ BDC C406, C414, C496, C498, GWC 627, 631

I. PURPOSE

1. This procedure outlines methods for safe handling and clean-up of chemical spills throughout the lab.

II. RESPONSIBILITY

1. It is the responsibility of all program staff to contribute to and comply with these procedures.
2. It is the responsibility of the facility manager to provide and maintain adequate materials needed for the clean-up of chemical spills.

III. PROCEDURES

1) Preventing a Spill

The possibility of a spill and preparation for handling it should be anticipated in setting up your

experiment. Appropriate precautions will alleviate many associated complications.

Before using a hazardous chemical:

- Familiarize yourself with the potential hazards of that chemical. Material Safety Data Sheets (MSDS's) are a valuable source of information. Each laboratory has a notebook of MSDS's of the hazardous chemicals used in that laboratory. Whenever you add a new chemical, you should add the MSDS sheet to that notebook.
- Evaluate the type of toxicity of the hazardous chemical (i.e., corrosive, irritant, sensitizer, carcinogen) and the possible routes of exposure (i.e., inhalation, skin absorption, ingestion, injection). Evaluate hazards of flammable and explosive chemicals.
- Select appropriate procedures to minimize exposure. Wear appropriate eye protection and protective apparel.
- Ask yourself, what is the worst that could happen? Ask yourself if you are prepared to handle such a situation. Do not underestimate risks, and consider substituting less hazardous materials, techniques, and equipment.

- Be prepared for accidents. Know what specific action you will take in the event of a chemical spill. Know the location of the laboratory spill kit, be familiar with the location of the nearest fire alarm and telephone, and know emergency telephone numbers.
- Have a knowledgeable colleague review your experimental design and safety procedures to judge the adequacy of the precautions and emergency steps.
- Purchase only the amount of hazardous material that will be used within a reasonable period, in the smallest container that is practical.
- Plan the transportation of hazardous materials to avoid heavy traffic areas and times. Use hazardous materials inside the chemical fume hood.
- Use secondary containers, metal cans, or plastic-coated bottles for storing and transporting.
- Do not place glass containers of chemicals on the floor.
- Take precautions to avoid fallen or leaking gas cylinders.

(2) What to do when a Spill Occurs

Whoever causes a minor spill must clean it up. However, some spills may be too large or too dangerous for laboratory personnel to handle, in which case ASU EH&S will work with you to arrange for a professional spill team to come in.

Minor Chemical Spill

- Alert people in the area that a spill has occurred.
- Use personal protective equipment (provided in the spill kits located in each laboratory) to protect yourself during the clean up. Avoid breathing vapors from the spill.
- Avoiding risks of injury or contamination, confine the spill to a small area. Prevent the spill from entering any drains.
- Neutralize/absorb the spill with shakers for acids, caustics, and solvents using the spill kits located in each laboratory.
- Collect residue, place in an appropriate container, label with a tag, and dispose of as hazardous waste. Schedule for a hazardous waste pickup using the EHS Assistant.
- Clean the spill area with water.

Major Chemical Spill

- Alert people in the lab that a spill has occurred and ask them to evacuate; close doors to the affected area.
- If spilled material is flammable, turn off ignition and heat sources if it can be done safely.
- Attend to injured or contaminated persons and remove them from exposure. If necessary, request help by calling the Campus Police (480-965-3456), or for life threatening emergencies call 911.

- If you feel you have been exposed to any hazardous material, report to ASU Medical for an evaluation.
- Contact ASU EH&S (480-965-1823) for cleanup assistance after caring to affected employees.
- If it can be done safely, block off any drains where the spill may enter.
- Have a person knowledgeable about the incident and laboratory standby to assist emergency personnel. If chemicals are known, provide MSDS' s for the emergency response team.

(3) Spill Kits

Each laboratory is equipped with spill kits which contains:

- Personal protective equipment: goggles and gloves that are chemical-resistant.
- Spill control pillows and wipes
- Disposal bags to collect the spilled material and contaminated clean-up materials.

Be aware of the location of spill kits. They should be checked on a regular basis.

Needle Stick Incident Protocol for Nielsen Lab

I. Introduction

The Nielsen laboratory will be working with recombinant bacteria and yeast. To minimize the risks of exposure to infectious materials, all personnel working in the Nielsen laboratory are required to take the Biosafety and Bloodborne Pathogen training offered by EH&S Biosafety. This training is required before work in the lab begins and annually thereafter. In addition, all personnel will be trained for their duties by senior lab personnel and informed of all potential risks associated with working in the laboratory. All personnel working with these materials will be offered the Hepatitis B vaccination free of charge as outlined in the ASU Bloodborne Pathogens Plan. If the worker declines the vaccination, a signed declination form will be kept on file.

II. Needlestick Procedures

Human blood products, cancer cell lines or other microorganisms

In the event of a needle-stick incident with possible exposure to bloodborne pathogens, human cancer cell lines or other microorganisms materials immediately follow these steps:

1. Gently squeeze the area to encourage gentle bleeding and reduce the possibility of exposure.
2. Wash exposed area with soap and water.
3. Report the incident to Prof. Nielsen at 5-4113
4. Seek medical attention immediately at:

ASU Health Services (Tempe)
451 E. University Dr.
Tempe, AZ 85287-2104
(480) 965-3349

5. If ASU Health Services is closed, emergency care may be obtained at the nearest emergency room and reported to ASU Health Services and EH&S Biosafety/Biosecurity the next business day. **ASU After Hours Medical Advice at 480-965-3349**. The nearest emergency room from ASU Tempe campus is:

Tempe St. Luke's Hospital
1500 South Mill Avenue
Tempe, AZ 85281
(480) 784-5500

6. Prof. Nielsen will report the incident to EH&S. The EH&S direct phone number is: (480) 965-1823.
7. Fill out the [Report of Injury](#). The form should be filled out within 24 hours of an accident or injury. Privacy and confidentiality procedures will be followed.
8. Post-exposure evaluation is required as determined by the medical provider.

In addition, whenever someone is injured or becomes ill from work-related incidents, the Arizona Department of Administration (ADOA) requires the following forms to be completed within 48 hours in

order to process Worker's Compensation Claims:

- Employer Report of Injury:
<https://www.asu.edu/hr/forms/wcformspacket.pdf>
- EHS Incident Report:
<https://cfo.asu.edu/incident-reporting>

Prof. Nielsen will report all accidents and injuries to EH&S Biosafety/Biosecurity. Federal, state, and local agencies may also need to be notified depending on the nature of the accident/injury. If the project involves recombinant DNA, the [IBC](#) will be required to report any significant problems with or violations of the NIH [Guidelines for Research with Recombinant or Synthetic DNA Molecules](#) and any significant research-related accidents or illnesses to the NIH within 30 days.

I. Post-Exposure Evaluation and Follow-Up

Following a report of an exposure incident, the employee will be provided a confidential medical evaluation and follow-up. This follow-up must include documentation of the route(s) of exposure and the circumstances under which the exposure incident occurred, identification and testing of the source individual's blood if available, collection and testing of the employee's blood, post-exposure prophylaxis (when medically indicated), evaluation of reported illnesses, and counseling. ASU will provide this evaluation and follow-up through ASU Health Services or contracted health care providers at no cost to the employee.

A. Blood Collection and Testing

The exposed employee's blood must be collected no later than 10 calendar days after the exposure incident. Serological testing for HIV, HBV, and HCV will be performed after consent is obtained; a healthcare professional's written opinion will be made available within 15 days after completion of the evaluation. Testing must be completed no later than 30 calendar days after the exposure incident. No later than 18 months after the date of the exposure incident, the employee will be retested. If an employee chooses not to complete the testing, that employee may jeopardize the availability of worker's compensation benefits from the Arizona Department of Administration, [Risk Management Division](#).

B. Information Provided to the Health Care Provider

The health care professional responsible for the employee's hepatitis B vaccination will be provided a copy of the [OSHA Bloodborne Pathogens Standard](#). The health care professional evaluating an employee after an exposure incident will be provided the following information:

- A copy of the [OSHA Bloodborne Pathogens Standard](#).
- A description of the exposed employee's duties as they relate to the exposure incident.
- Documentation of the route(s) of exposure and circumstances under which exposure occurred.
- Results of the source individual's blood testing, if available.

C. Evaluation of Incident

The circumstances surrounding the exposure incident will be investigated immediately by Prof. Nielsen. Information regarding the exposure incident, source material, and employee

vaccination status will be provided to ASU Health Services and/or the employee's health care provider. Site-specific procedures will be reevaluated and revised by Prof. Nielsen as necessary to prevent recurrences of similar incidents. EH&S Biosafety/Biosecurity is available to assist the Nielsen laboratory personnel with evaluating the following:

1. Engineering controls and work practices used at the time of the exposure.
2. A description of any devices being used (e.g., sharps, centrifuge, blender).
3. Protective equipment or clothing worn at the time of the exposure incident.
4. A review of the procedures being performed at the time of the incident.
5. A review of the employee's training record.

II. Documentation and Recordkeeping

A. Medical Recordkeeping

ASU Health Services will establish and maintain an accurate record for each employee with occupational exposure, in accordance with 29 CFR § 1910.20³ The record shall include:

- The name and social security number of the employee.
- A copy of the employee's hepatitis B vaccination status including the dates of all the hepatitis B vaccinations and any medical records relative to the employee's ability to receive vaccination.
- A copy of all results of examinations, medical testing, and follow-up procedures required.
- The copy of the healthcare professional's written opinion as required.
- A copy of the information provided to the healthcare professional as required.

ASU Health Services will ensure that employee medical records required are kept confidential and not disclosed or reported without the employee's express written consent to any person within or outside the workplace except as required by the standard or as may be required by law. ASU Health Services will maintain the records required for at least the duration of employment plus thirty years in accordance with 29 CFR § 1910.20⁴.

B. Employee Records

ASU is required to establish and maintain an accurate record for each employee with occupational exposure, in accordance with 29 CFR 1910.1020. This record is maintained by ASU Health Services and includes:

1. The name and social security number of the employee.
2. A copy of the employee's hepatitis B vaccination status including the dates of all the hepatitis B vaccinations and any medical records relative to the employer's ability to receive vaccination.
3. All medical records pertaining to an exposure incident and follow-up evaluation. All documentation will be held under strict confidentiality guidelines.

C. Sharps Injury Log

ASU is required to establish and maintain a sharps injury log (see [Appendix B](#)) for the recording of percutaneous injuries from contaminated sharps. The information in the sharps injury log shall be recorded and maintained in such manner as to protect the confidentiality of the injured employee. The sharps injury log will be maintained by Prof. Nielsen and a copy will be provided

monthly to EH&S Biosafety/Biosecurity at biosafety@asu.edu. The sharps injury log will contain the following information:

1. The type and brand of device involved in the incident.
2. The laboratory in which the exposure occurred.
3. An explanation of how the incident occurred and personnel involved.

D. Documentation of Updated Safe Practices

Consideration of changes in technology that reduce or eliminate exposure must be evaluated and documented annually including solicitation of input from non-managerial staff.

References:

1. Strauss, D. C.; Thomas, J. M. Transmission of Donor Melanoma by Organ Transplantation. *Lancet Oncol.* 2010, 11 (8), 790–796.
2. SCANLON, E. F.; HAWKINS, R. A.; FOX, W. W.; SMITH, W. S. FATAL HOMOTRANSPLANTED MELANOMA: A CASE REPORT. *Cancer* 1965, 18, 782–789.

Standard Operating Procedure

Benzene

Print a copy and insert into your
Laboratory Safety Manual and Chemical Hygiene Plan.
 Refer to instructions for assistance.

School / Department:	SEMTE		
SOP Preparation Date:	5/11/2016	SOP Approval Date:	Click here to enter a date.
Principal Investigator:	David Nielsen		
Lab Manager Name:	Click here to enter text.		
Laboratory Phone:	Click here to enter text.	Office Phone:	5-4113
Emergency Contact:	Fred Pena	Contact Phone:	5-4122
Laboratory locations covered by this SOP (building / room number):			
BDC C414B			

Type of SOP:	<input type="checkbox"/> Process	<input checked="" type="checkbox"/> Hazardous Chemical	<input type="checkbox"/> Hazardous Class
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Purpose

Benzene is an OSHA regulated carcinogen. It is a mutagenic flammable liquid. It is an organic solvent and precursor in the production of drugs, plastics, synthetic rubber, polymers, explosives, and dyes.

Physical and Chemical Properties / Definition of Chemical Group

CAS:	71-43-2	
Class:	Flammable liquid, regulated carcinogen, mutagen	
Molecular Formula:	C ₆ H ₆	
Form (physical state):	Liquid	
Color:	Colorless	
Boiling Point:	80.0 – 80.2°C (176.0 – 176.4°F)	

Potential Hazards / Toxicity

Potential Health Effects

<i>May cause genetic defects, may cause cancer.</i>	
Target Organs:	Blood, eyes, female reproductive system, bone marrow
Inhalation:	May be harmful if inhaled, causes respiratory tract irritation.
Skin:	May be harmful if absorbed through skin, causes skin irritation.
Eyes:	May cause eye damage, causes eye irritation.
Ingestion:	May be harmful if swallowed. Aspiration hazard if swallowed – can enter lungs and cause damage to lung tissue.

Personal Protective Equipment (PPE)

Respiratory Protection

Where risk assessment shows air-purifying respirators are appropriate, use a full-face respirator with multi-purpose combination (US) respirator cartridges as a backup to engineering controls.

Respirators should be used only under any of the following circumstances:

- As a last line of defense (i.e., after engineering and administrative controls have been exhausted).
- When Permissible Exposure Limit (PEL) has exceeded or when there is a possibility that PEL will be exceeded.
- Regulations require the use of a respirator.
- An employer requires the use of a respirator.
- There is potential for harmful exposure due to an atmospheric contaminant (in the absence of PEL)
- As PPE in the event of a chemical spill clean-up process

Lab personnel intending to use/wear a respirator mask must be trained and fit-tested by EH&S. This is a regulatory requirement.

(http://www.asu.edu/uagc/EHS/documents/asu_respiratory_protection_plan.pdf)

Hand Protection

- Silver shield or Viton gloves are recommended.

NOTE: Consult with your preferred glove manufacturer to ensure that the gloves you plan on using are compatible with Hydrofluoric acid.

Refer to glove selection chart from the links below:

http://www.ansellpro.com/download/Ansell_8thEditionChemicalResistanceGuide.pdf

OR

<http://www.allsafetyproducts.biz/page/74172>

OR

<http://www.showabestglove.com/site/default.aspx>

OR

<http://www.mapaglove.com/>

Eye Protection

- Wear NIOSH approved safety glasses or goggles

Skin & Body Protection

- Flame resistant or retardant lab coats should be worn – lab coats must be buttoned to their full length and coat sleeves must be of a sufficient length to prevent skin exposure while wearing gloves.
- Full-length pants
- Close-toed shoes – the area of skin between the shoe and ankle should not be exposed

Hygiene Measures

Handle in accordance with good industrial hygiene and safety practice. Avoid contact with skin, eyes, and clothing. Wash hands before breaks and immediately after handling the product.

Engineering Controls

All operations involving benzene and dilutions should be carried out in a ventilated enclosure fume hood to keep airborne concentrations below recommended exposure limits. Chemical fume hoods used as containment areas for particularly hazardous chemicals must have a face velocity of 100 cfm, averaged over the face of the hood and must be certified annually. Laboratory rooms must be at negative pressure with respect to the corridors and external environment. The laboratory/room door must be kept closed at all times.

First Aid Procedures

If inhaled... Move to fresh air. If the person is not breathing, give artificial respiration and call 911 from a campus phone or (480) 965-3456. Call EH&S at (480) 965-1823.

In case of skin contact... Minor skin contact requires washing with soap and water. Soaking or flushing contaminated areas of the skin with water for periods up to 15 minutes is required if a large area comes into contact with the chemical, or if prolonged contact occurs. Contaminated clothing may hold the chemicals in contact with the skin without being immediately noticed. Many chemicals are absorbed through the skin, and dermatitis may later appear on skin which appears to be clean.

In case of eye contact... In the event of eye contact, the eye should be immediately flushed with water. If the chemical is very irritating, it is likely that the affected individual will require

assistance to hold the eye open during the flushing. Use nearest emergency eyewash immediately. Call 911 from a campus phone or (480) 965-3456 from a cell phone. Call EH&S at (480) 965-1823.

If swallowed... Give milk or water to induce vomiting if conscious. Never give anything by mouth to an unconscious person. Get medical attention immediately, call 911 from a campus phone or (480) 965-3456 from a cell phone. Call EH&S at (480) 965-1823.

Special Storage & Handling Requirements

Storage

- Ensure the container is tightly closed at all times.
- Store in a dry and well-ventilated place.
- Use explosion-proof equipment.
- Keep away from sources of ignition – no smoking.
- Containers which are opened must be carefully resealed and kept upright to prevent leakage
- Signage is required for the container, designated work area and storage.
- Sign wording must state “Danger, Cancer Hazard.”

Handling

- Avoid contact with skin and eyes.
- Avoid inhalation of vapor and mist.
- Take measures to prevent the build-up of electrostatic charge.
- Ensure you are wearing the following minimum PPE: NIOSH approved safety glasses or goggles, lab coat, full length pants, close-toed shoes

Spill and Accident Procedure

Personal precautions

Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Do not attempt clean-up without minimum PPE. If chemical spill on body or clothes – remove clothing and rinse body thoroughly in emergency shower for at least 15 minutes, seek medical attention, and notify supervisor and EH&S at (480) 965-1823. If chemical splash into eyes – immediately rinse eyeball and inner surface of eyelid with water from the emergency eyewash station for 15 minutes by forcibly holding eye open, seek medical attention, and notify supervisor and EH&S at (480) 965-1823.

Environmental precautions

Prevent further leakage or spillage – if safe to do so. Do not allow product to enter drains.

Methods and materials for containment and clean-up

Consider material compatibility prior to clean-up. Verify spill kit is available.

1. Immediately assess amount spilled, follow posted ASU Emergency Response Guide procedures for hazardous materials incidents.
2. If a chemical exposure has occurred, a fellow lab worker shall call 9-1-1 and EH&S at (480) 965-1823.
3. Don compatible gloves and other protective PPE if not already being worn.
4. Secure / restrict access to the area of the spill to prevent spread of the chemical.
5. Use the available spill kit to stop and contain the spill. Bag the collected material.
6. Label and tag as hazardous waste and submit a pick-up request to EH&S using EHS Assistant.

Decontamination / Waste Disposal Procedure

All waste streams containing benzene will be collected as hazardous waste. Decontaminate work space with 70-75% ethanol. Wash hands and arms with soap and water after finished. Contaminated pipet tips, eppendorf tubes, and gloves should be discarded as hazardous waste in accordance with ASU EH&S waste disposal procedures.

Label waste

- Attach a completed ASU Hazardous Waste tag to all waste containers as soon as the first drop of waste is added to the container.

Store waste

- Store hazardous waste in closed containers, in secondary containment and in a designated storage location.
- Double-bag dry waste using sealable transparent bags.
- Waste must be under the control of the person generating and disposing of it.

Dispose of waste

- Dispose of regularly generated chemical waste within 90 days.
- Use EHS Assistant online hazardous waste pick-up request system.
- Contact ASU EH&S at (480) 965-1823 with questions.

Safety Data Sheet (SDS) Location

Inside BDC C496A, on top of fridge in yellow binders.

Protocol / Procedure

Laboratory-specific procedures

All dispensing of benzene should take place in the chemical hood. Use only small volumes. If small spills occur, close hood sash and allow to evaporate.

Documentation of Training

- Prior to conducting any work with this material, Principal Investigator or designee must provide to his/her laboratory personnel specific to the hazards involved in working with this substance, work area decontamination, and emergency procedures.
- The Principal Investigator must provide his/her laboratory personnel with a copy of this SOP and a copy of the MSDS provided by the manufacturer.
- The Principal Investigator must ensure that his/her laboratory personnel have attended appropriate/required laboratory safety training or refresher training within the last one year.

I have read and understand the content of this SOP.

Employee Name	ASU Affiliate No.	Signature	Date
David Nielsen	1202345876		1/3/2022
Click here to enter text.	Click here to enter text.		Click here to enter a date.
Click here to enter text.	Click here to enter text.		Click here to enter a date.
Click here to enter text.	Click here to enter text.		Click here to enter a date.
Click here to enter text.	Click here to enter text.		Click here to enter a date.
Click here to enter text.	Click here to enter text.		Click here to enter a date.
Click here to enter text.	Click here to enter text.		Click here to enter a date.

Standard Operating Procedure

Sodium azide

Print a copy and insert into your
Laboratory Safety Manual and Chemical Hygiene Plan.
 Refer to instructions for assistance.

School / Department:	SEMTE		
SOP Preparation Date:	1/24/2018	SOP Approval Date:	1/1/2418
Principal Investigator:	David Nielsen		
Lab Manager Name:	Fred Pena		
Laboratory Phone:	N/A	Office Phone:	(480) 965-4122
Emergency Contact:	David Nielsen	Contact Phone:	(480) 965-4113
Laboratory locations covered by this SOP (building / room number): BDC C414B			

Type of SOP: Process Hazardous Chemical Hazardous Class

Purpose

Sodium azide is commonly used in research labs as a preservative in diluted solutions of 0.1% to 2% and in organic synthesis for the synthesis of triazoles, tetrazoles, aziridines, and primary amines.

Physical and Chemical Properties / Definition of Chemical Group

CAS:	26628-22-8	
Class:	Acute toxin, Water reactive, Potentially explosive chemical	
Molecular Formula:	NaN ₃	
Form (physical state):	Solid	
Color:	White	
Boiling Point:	275 °C	

Potential Hazards / Toxicity

Potential Health Effects

Target Organs:	Heart, Central nervous system, Brain
Inhalation:	May be harmful if inhaled. May cause respiratory tract irritation.
Skin:	May be fatal if absorbed through skin. May cause skin irritation.
Eyes:	May cause eye irritation.
Ingestion:	May be fatal if swallowed.

Personal Protective Equipment (PPE)

Respiratory Protection

Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Respirators should be used only under any of the following circumstances:

- As a last line of defense (i.e., after engineering and administrative controls have been exhausted).
- When Permissible Exposure Limit (PEL) has exceeded or when there is a possibility that PEL will be exceeded.
- Regulations require the use of a respirator.
- An employer requires the use of a respirator.
- There is potential for harmful exposure due to an atmospheric contaminant (in the absence of PEL)
- As PPE in the event of a chemical spill clean-up process

Lab personnel intending to use/wear a respirator mask must be trained and fit-tested by EH&S. This is a regulatory requirement.

<http://www.asu.edu/ehs/documents/asu-respiratory-protection-plan.pdf>

Hand Protection

Handle with gloves. Use proper glove removal technique to avoid skin contact with this product. Nitrile gloves are recommended.

NOTE: Consult with your preferred glove manufacturer to ensure that the gloves you plan on using are compatible with Sodium azide.

Refer to glove selection chart from the links below:

http://www.ansellpro.com/download/Ansell_8thEditionChemicalResistanceGuide.pdf

OR

<http://www.allsafetyproducts.biz/page/74172>

OR

<http://www.showabestglove.com/site/default.aspx>

Eye Protection

- Wear chemical splash goggles or a face shield to protect from splash hazards and chemical vapors.

Skin & Body Protection

- Lab coat
- Full-length pants
- Closed-toe rubber or leather shoes

Hygiene Measures

Avoid contact with skin, eyes, and clothing. Wash hands before breaks and immediately after handling the product.

Engineering Controls

Handle using a certified chemical fume hood with adequate ventilation.

First Aid Procedures

If inhaled... Move to fresh air. If the person is not breathing, give artificial respiration. DO NOT use mouth to mouth contact. If breathing is difficult, give oxygen. Call 911 from a campus phone or (480) 965-3456. Call EH&S at (480) 965-1823.

In case of skin contact... Remove all contaminated clothing. Immediately (within seconds) flush affected area for FIFTEEN (15) minutes. Call 911 from a campus phone or (480) 965-3456 from a cell phone. Call EH&S at (480) 965-1823.

In case of eye contact... Remove any contact lenses. Use nearest emergency eyewash immediately for at least 15 minutes. Call 911 from a campus phone or (480) 965-3456 from a cell phone. Call EH&S at (480) 965-1823.

If swallowed... DO NOT INDUCE VOMITING. Never give anything by mouth to an unconscious person. Rinse mouth with water. Call 911 from a campus phone or (480) 965-3456 from a cell phone. Call EH&S at (480) 965-1823.

Special Storage & Handling Requirements

Storage

- Ensure the container is tightly closed at all times.
- Keep in a cool, dry, well-ventilated area away from incompatible materials and conditions.
- Do not store on metal shelves or use metal items (spatulas) to handle Sodium azide.

- Keep away from heat, air, light, and moisture.
- Do not allow product to get in contact with water during storage.
- Sodium azide and all other acutely toxic materials should be stored in a secondary container in a designated area away from other chemicals.
- Store away from metals, acids, carbon disulfide, bromine, chromyl chloride, sulfuric acid, nitric acid, hydrazine and dimethyl sulfate.

Handling

- The lab where the material is being handled has an approved / certified emergency eyewash and safety shower.
- Ensure you are wearing the following minimum PPE: tightly fitting safety goggles, lab coat, full length pants, closed-toe rubber or leather shoes, nitrile gloves.
- Lab emergency contact information must be readily posted. Easy access to a cellular phone or land line is readily available.
- Avoid contact with skin, eyes, and clothing.
- Avoid formation of dusts and aerosols.
- Provide appropriate exhaust ventilation at places where dust is formed.

Spill and Accident Procedure

Personal precautions

Wear respiratory protection. Avoid dust formation. Avoid breathing vapors, mist, dust or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Do not attempt clean-up without minimum PPE.

Environmental precautions

Prevent further leakage or spillage – if safe to do so. Do not allow product to enter drains. Discharge into the environment must be avoided.

Methods and materials for containment and clean-up

Consider material compatibility prior to clean-up. Verify spill kit is available. Pick up and arrange disposal without creating dust. Sweep up and shovel. Do not flush with water. Keep in suitable, closed containers for disposal.

1. Immediately assess amount spilled, follow posted ASU Emergency Response Guide procedures for hazardous materials incidents.
2. If a chemical exposure has occurred, a fellow lab worker shall call 9-1-1 and EH&S at (480) 965-1823.
3. Don compatible gloves and other protective PPE if not already being worn.
4. Secure / restrict access to the area of the spill to prevent spread of the chemical.
5. Use the available spill kit to stop and contain the spill. Bag the collected material.
6. Label and tag as hazardous waste and submit a pick-up request to EH&S using EHS Assistant.

Decontamination / Waste Disposal Procedure

Label waste

- Attach a completed ASU Hazardous Waste tag to all waste containers as soon as the first drop of waste is added to the container.

Store waste

- Store hazardous waste in closed containers, in secondary containment and in a designated storage location.
- Double-bag dry waste using sealable transparent bags.
- Waste must be under the control of the person generating and disposing of it.

Dispose of waste

- Dispose of regularly generated chemical waste within 90 days.
- Use EHS Assistant online hazardous waste pick-up request system.
- Contact ASU EH&S at (480) 965-1823 with questions.

Protocol / Procedure

Laboratory-specific procedures

Sodium azide may be stored with other chemicals overhead in the benchtop cabinets, but it must be sealed in a transparent plastic bag as secondary containment. Neither the bag nor bottle should ever be opened outside of a chemical fume hood. It is highly recommended that pure sodium azide only be used to make stock solutions, and that those solutions enjoy the same respect. Work generating or using sodium azide products must be undertaken with proper protective equipment, including safety glasses, lab coat, and gloves. All lab materials containing or coming into contact with sodium azide that would be disposed of in the biohazard waste (used gloves, plates, empty tubes from sodium azide-induced cultures, etc.) must first be separately bagged to ensure that no handler comes into exposure as waste is being sterilized.

IMPORTANT NOTE: Any deviation from this SOP requires advance PI approval.

Documentation of Training

- Prior to conducting any work with this material, Principal Investigator or designee must provide to his/her laboratory personnel specific to the hazards involved in working with this substance, work area decontamination, and emergency procedures.
- The Principal Investigator must provide his/her laboratory personnel with a copy of this SOP and a copy of the MSDS provided by the manufacturer.
- The Principal Investigator must ensure that his/her laboratory personnel have attended appropriate/required laboratory safety training or refresher training within the last one year.

I have read and understand the content of this SOP.

Employee Name	ASU Affiliate No.	Signature	Date
David Nielsen	1202345876		1/3/2022
Click here to enter text.	Click here to enter text.		Click here to enter a date.
Click here to enter text.	Click here to enter text.		Click here to enter a date.
Click here to enter text.	Click here to enter text.		Click here to enter a date.
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Click here to enter text.	Click here to enter text.		Click here to enter a date.

Standard Operating Procedure

Thiols, Mercaptans, and Other Stench Chemicals

This is an SOP template and is not complete until: 1) lab specific information is entered into the box below 2) lab specific protocol/procedure is added to the protocol/procedure section and 3) SOP has been signed and dated by the PI and relevant lab personnel.

Print a copy and insert into your
Laboratory Safety Manual and Chemical Hygiene Plan.
Refer to instructions for assistance.

School / Department:	SEMTE		
SOP Preparation Date:	3/8/2021	SOP Approval Date:	Click here to enter a date.
Principal Investigator:	David Nielsen		
Lab Manager Name:	Fred Pena		
Laboratory Phone:	None	Office Phone:	5-4113
Emergency Contact:	David Nielsen	Contact Phone:	617-233-3718
Laboratory locations covered by this SOP (building / room number):			
BDC C414B			

Type of SOP: Process Hazardous Chemical Hazardous Class

Stench chemicals have an extremely foul smell that is detectable by the human nose in amounts as little as 1.4×10^{-4} ppb,¹ depending on the compound. While the stench may not be known to have toxic effects, it can still negatively impact laboratory workers and people outside the laboratory (even outside the building) by inducing nausea and/or vomiting, and causing reports of gas leaks or sewage problems. The odors have even been responsible for building evacuations at ASU. Therefore, handling and disposal of stench compounds should be carefully controlled in order to minimize the release of odors.

Stench chemicals

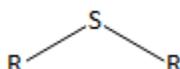
- thiols



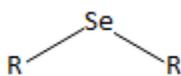
include:

(mercaptans)

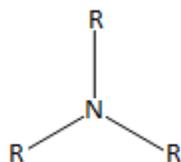
- sulfides



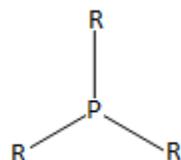
- selenides



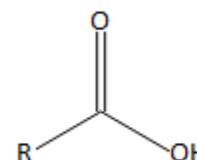
- amines



- phosphines



- carboxylic acids with short aliphatic hydrocarbon chains, such as butyric and valeric acid



“R” can include any organic chains or structures.

Purpose

The chemicals in these stench families can serve a wide variety of purposes in the lab.

Physical and Chemical Properties / Definition of Chemical Group

CAS:	varies	<div style="text-align: center;">varies</div> 
Class:	varies	
Molecular Formula:	RSH, R ₂ S, R ₂ Se, R ₃ N, or R ₃ P where R = aliphatic hydrocarbon chain or H; RCOOH where R = short aliphatic hydrocarbon chain	
Form (physical state):	varies	
Color:	varies	
Boiling Point:	varies	

Preceding information may change based on specific guidance for the hazardous material.

Potential Hazards / Toxicity

Potential Health Effects	
Target Organs:	varies
Inhalation:	varies
Skin:	varies
Eyes:	varies
Ingestion:	varies

Preceding information may change based on specific guidance for the hazardous material.

Personal Protective Equipment (PPE)

Respiratory Protection

Respirators should be used only under any of the following circumstances:

- As a last line of defense (i.e., after engineering and administrative controls have been exhausted).
- When Permissible Exposure Limit (PEL) has exceeded or when there is a possibility that PEL will be exceeded.
- Regulations require the use of a respirator.
- An employer requires the use of a respirator.
- There is potential for harmful exposure due to an atmospheric contaminant (in the absence of PEL).
- As PPE in the event of a chemical spill clean-up process.

Lab personnel intending to use/wear a respirator mask must be trained and fit-tested by EH&S. This is a regulatory requirement.

<http://www.asu.edu/ehs/documents/asu-respiratory-protection-plan.pdf>

Preceding information may change based on specific guidance for the hazardous material.

Hand Protection

NOTE: Consult with your preferred glove manufacturer to ensure that the gloves you plan on using are compatible with the specific stench chemical you are using. If the stench chemical in use is known for certain to *not* permeate the gloves that you are using for an hour or more (“no data” is not sufficient), then layering of multiple pairs of gloves and removing one layer at a time may replace glove changes during handling. However, gloves must be changed and hands washed as soon as the handling is complete and the container of stench chemical is put away.

Refer to glove selection chart from the links below:

http://www.ansellpro.com/download/Ansell_8thEditionChemicalResistanceGuide.pdf

OR

<http://www.allsafetyproducts.biz/page/74172>

OR

<http://www.showabestglove.com/site/default.aspx>

Preceding information may change based on specific guidance for the hazardous material.

Eye Protection

- Wear chemical splash goggles to protect from splash hazards and chemical vapors. A face shield may be worn over the goggles if needed.

Preceding information may change based on specific guidance for the hazardous material.

Skin & Body Protection

- Lab coat; add a natural rubber apron if needed
- Full-length pants
- Close-toe rubber or leather shoes (unless a different shoe material is specifically required)

Preceding information may change based on specific guidance for the hazardous material.

Hygiene Measures

Avoid contact with skin, eyes, and clothing. Wash hands before breaks and immediately after handling the product.

Preceding information may change based on specific guidance for the hazardous material.

Engineering Controls

All operations involving stench chemicals and dilutions should be carried out in a ventilated enclosure fume hood to keep airborne concentrations below recommended exposure limits. Chemical fume hoods used as containment areas for particularly hazardous chemicals must have a face velocity of 100 cfm, averaged over the face of the hood and must be certified annually. The fume hood sash should be kept as low as possible. Laboratory rooms must be at negative pressure with respect to the corridors and external environment. The laboratory/room door must be kept closed at all times.

If the stench chemical is a solid, it must be weighed and used entirely inside the fume hood. After use, the scale and its glass covering, handles, and buttons should be wiped down with bleach (aqueous sodium hypochlorite, or NaClO) or other compatible oxidant

(i.e. the oxidant should not have any violent reaction with the stench chemical). Gloves should be replaced before carrying the scale out of the fume hood.

If the stench chemical is a liquid, all transfers should be performed using a syringe or cannula. Use the volume markings on the syringe and the liquid's density to determine the amount; do not weigh it on a scale. Never pour or pipette these liquids or their dilutions. Any re-usable cannulas, needles, or syringes should be cleaned with bleach or another compatible oxidant prior to re-use.

Avoid using excess amounts of stench chemicals. The reaction mixture must be handled as a stench chemical until all unreacted stench chemical is thoroughly removed from the mixture.

Depending on the potency of the stench chemical in question, a cold finger trap or bleach trap (examples illustrated below, Figures 1 & 2) must be used to prevent stench chemical fumes from being released into the fume hood. The process must be evaluated by the PI or other responsible party first to ensure that none of the components in the experimental setup are incompatible with bleach.

Bleach (aqueous sodium hypochlorite, or NaClO) is capable of oxidizing thiols, sulfides, selenides, phosphines, and some amines to their less-smelly counterparts (such as sulfoxides, sulfones, phosphine oxides, etc.).^{2,3} Bleach can also oxidize carboxylic acids through decarboxylation.⁴

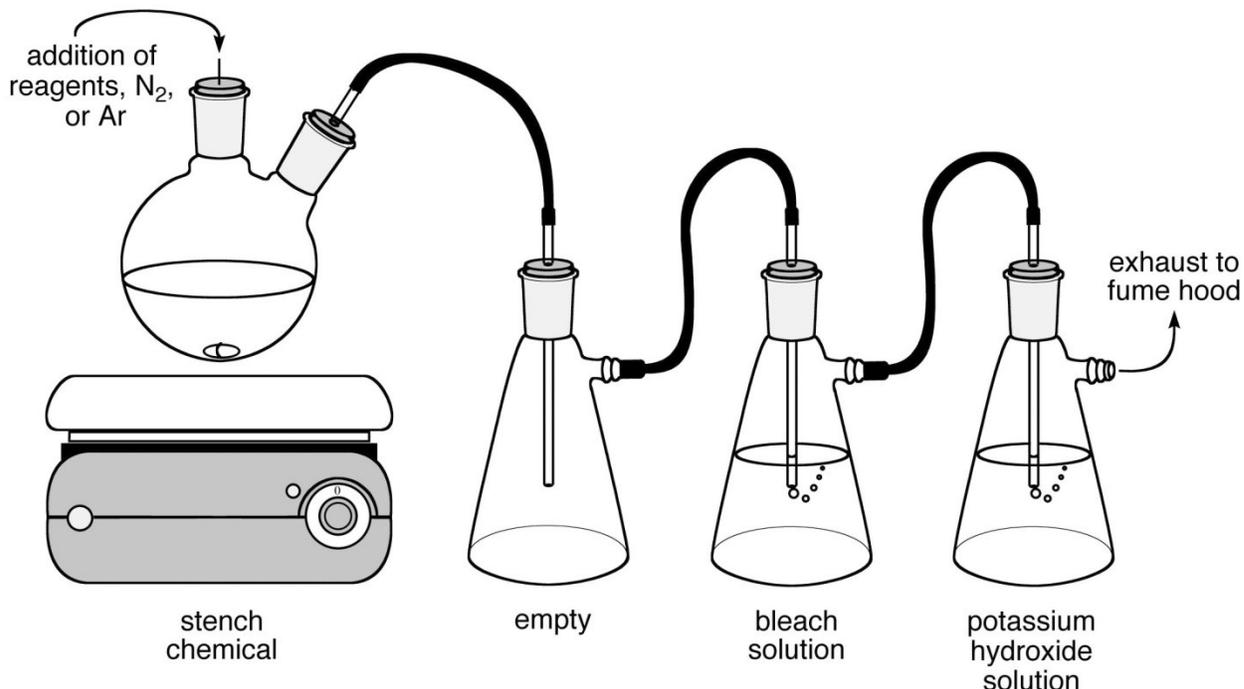


Figure 1.² Exhaust from a system that contains stench chemical fumes is passed through bleach. The hydrochloric acid (HCl) vapor that is generated as a by-product of oxidation is neutralized by passing through a strong base solution (0.01 M should be sufficient).

Both the oxidation and neutralization reactions are exothermic and will generate heat.² Depending on the amount of stench chemicals passing through the flasks, ice baths may be required in order to control the temperature.

Clorox brand bleach is typically 5-10% sodium hypochlorite by weight.⁶ Other brands may have lower concentrations and therefore may be less effective in bleach traps.⁷ Consult the brand's MSDS/SDS for concentration prior to use. Do not attempt to acidify the bleach solution; dropping the pH of the bleach solution will cause off-gassing of toxic Cl_2 gas.⁷

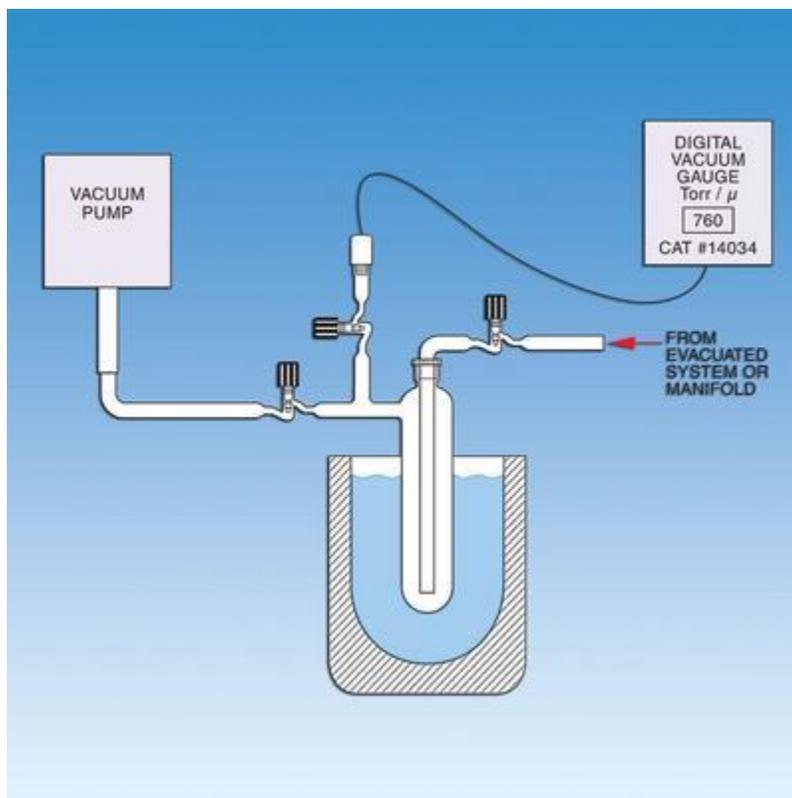


Figure 2.⁵ Exhaust from a system that contains stench chemical fumes is passed through a cold finger chamber surrounded by a dewar, which should be cooled to at least -78°C (it can be filled with dry ice in acetone, or liquid nitrogen). The vacuum gauge is optional, and the air exiting the cold finger may be exhausted into the fume hood if vacuum is not needed.

The cold finger setup is ideal in cases where an experimental component may be incompatible with bleach. The portion where the stench chemicals are trapped must be emptied into a stench waste container and rinsed out with a compatible oxidant. Rinsings should also go into a stench waste container, although not necessarily into the same container as the pure stench chemical; be cautious of exothermic reactions between the oxidant and the stench chemical.

If any mixture containing a stench chemical is to be concentrated under reduced pressure (by use of a rotovap, for example), the cold finger trap must be used in between the vacuum pump and the vacuum take-off of the evaporative device. This cold finger is

in addition to the normal condenser, and does not replace it. The entire apparatus must be located inside the fume hood, and cleaned with a compatible oxidant after use.

Preceding information may change based on specific guidance for the hazardous material.

First Aid Procedures

If inhaled... Move to fresh air. If the person is not breathing, give artificial respiration. Avoid mouth to mouth contact. Call 911 from a campus phone or (480) 965-3456. Call EH&S at (480) 965-1823.

Preceding information may change based on specific guidance for the hazardous material.

In case of skin contact... Remove all contaminated clothing. Immediately (within seconds) flush affected area for FIFTEEN (15) minutes. Call 911 from a campus phone or (480) 965-3456 from a cell phone. Call EH&S at (480) 965-1823.

Preceding information may change based on specific guidance for the hazardous material.

In case of eye contact... Use nearest emergency eyewash immediately. Call 911 from a campus phone or (480) 965-3456 from a cell phone. Call EH&S at (480) 965-1823.

Preceding information may change based on specific guidance for the hazardous material.

If swallowed... DO NOT INDUCE VOMITING. Never give anything by mouth to an unconscious person. Call 911 from a campus phone or (480) 965-3456 from a cell phone. Call EH&S at (480) 965-1823.

Preceding information may change based on specific guidance for the hazardous material.

Special Storage & Handling Requirements

Storage

- Ensure the stench chemical container is tightly closed at all times.
- The neck of the stench chemical container must be wrapped with parafilm, Teflon tape, electrical tape, or other compatible sealing tape.
- The outside of containers must be wiped down with bleach (or other compatible oxidant) after each use.
- If the stench chemical container still emits an odor after being wiped down and having its neck wrapped, then it must be placed inside a secondary container (such as a large jar with a lid) and the neck of the secondary container must be sealed with a

compatible tape. If the secondary container is not transparent (colorless glass), then it must be properly labeled.

Preceding information may change based on specific guidance for the hazardous material.

Handling

- The lab where the material is being handled has an approved / certified emergency eyewash and safety shower.
- Ensure you are wearing the following minimum PPE: tightly fitting safety goggles and face shield, lab coat & natural rubber apron, full length pants, close-toe rubber or leather shoes, gauntlet style (or arm length) natural rubber gloves over a pair of nitrile gloves.
- Lab emergency contact information must be readily posted. Easy access to a cellular phone or land line is readily available.
- Any equipment needed for the use of the stench chemical (pipettes, extra gloves, etc.) must be placed inside the fume hood so that it can be reached without removing contaminated gloves from the fume hood area.
- Great care must be taken to reduce the spread of stench chemicals during the process. From the moment that the stench chemical bottle is picked up, avoid touching any handles, valves, or surfaces unnecessarily. Adjust gas lines and fume hood sash height prior to the process.
- Any item that was *touched or used at any point* during the process (including gloves, paper towels, weighing paper, equipment handles, the fume hood sash, etc.) must be considered to be contaminated with the stench chemical, and should be cleaned or disposed of accordingly.
- All items, including sealed containers, must be decontaminated with bleach (or another compatible oxidant) before they may be removed from the fume hood.

Preceding information may change based on specific guidance for the hazardous material.

Spill and Accident Procedure

Personal precautions

Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Do not attempt clean-up without minimum PPE.

Environmental precautions

Prevent further leakage or spillage – if safe to do so. Do not allow product to enter drains.

Methods and materials for containment and clean-up

Consider material compatibility prior to clean-up. Verify spill kit is available.

1. Immediately assess amount spilled, follow posted ASU Emergency Response Guide procedures for hazardous materials incidents.
2. If a chemical exposure has occurred, a fellow lab worker shall call 9-1-1 and EH&S at (480) 965-1823.
3. Don compatible gloves and other protective PPE if not already being worn.
4. Secure / restrict access to the area of the spill to prevent spread of the chemical.
5. Use the available spill kit to stop and contain the spill. Bag the collected material.
6. Wipe down affected areas with a compatible oxidant. For spills outside the fume hood, use only oxidants that do not require the use of a fume hood, such as 3% H₂O₂ with NaHCO₃ (avoid the use of bleach due to the HCl byproduct). Bag the wipes separately from the collected material.
7. Label and tag as hazardous waste and submit a pick-up request to EH&S using EHS Assistant.

Preceding information may change based on specific guidance for the hazardous material.

Decontamination / Waste Disposal Procedure

Label waste

- Attach a completed ASU Hazardous Waste tag to all waste containers as soon as the first drop of waste is added to the container.
- In the “Category” section on the Hazardous Waste tag, be sure to include “Stench” as a hazard.

Store waste

- All waste that was exposed to or contains stench chemicals, (including gloves, weighing paper, paper towels, etc.) must be placed in containers that are separate from other waste.
- All waste exposed to or containing stench chemicals (including sharps) must be put in air-tight containers. Containers cannot leave the fume hood until they are tightly closed.
- Store hazardous waste in closed containers, in secondary containment and in a designated storage location.
- Dry waste should be stored in a glass or plastic container that cannot be permeated or dissolved by any of the chemicals in the waste.
- Waste must be under the control of the person generating and disposing of it.

Dispose of waste

- Dispose of regularly generated chemical waste within 90 days.
- Use EHS Assistant online hazardous waste pick-up request system.
- Contact ASU EH&S at (480) 965-1823 with questions.

Preceding information may change based on specific guidance for the hazardous material.

Protocol / Procedure

Laboratory-specific procedures

Limit use of these chemical to only the hoods located in C496A and C414B. Only open bottle once in the hood and cap/seal before removing it.

IMPORTANT NOTE: Any deviation from this SOP requires advance PI approval.

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- 3) National Research Council. *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals*; National Academy Press: Washington, 1995.
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- 5) VWR. High Vacuum Trap System, Ace Glass Incorporated.
https://ca.vwr.com/store/catalog/product.jsp?product_id=11733826 (accessed Oct. 23, 2015).
- 6) The Clorox Company. Clorox® Regular-Bleach₁.
<https://www.thecloroxcompany.com/downloads/msds/bleach/cloroxregular-bleach12015-06-12.pdf> (accessed Oct. 23, 2015).
- 7) Oregon State University. Fact Sheet: Disinfection Using Chlorine Bleach.
<http://oregonstate.edu/dept/larc/sites/default/files/pdf/chlorine-fact-sheet.pdf> (accessed Oct. 23, 2015).

Documentation of Training

- Prior to conducting any work with this material, Principal Investigator or designee must provide to his/her laboratory personnel specific to the hazards involved in working with this substance, work area decontamination, and emergency procedures.
- The Principal Investigator must provide his/her laboratory personnel with a copy of this SOP and a copy of the Safety Data Sheet (SDS) provided by the manufacturer.
- The Principal Investigator must ensure that his/her laboratory personnel have attended appropriate/required laboratory safety training or refresher training within the last one year.



I have read and understand the content of this SOP.

Employee Name	ASU Affiliate No.	Signature	Date
David Nielsen	1202345876		1/3/22
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