LSU Health NEW ORLEANS

Laboratory Safety Training

Updated 31 May 2019

LSU Health NEW ORLEANS

Outline

- Background and Purpose
- Housekeeping and Fire Safety
- Chemical Safety
- Biological Safety
- Radiation Safety
- Personal Protective Equipment (PPE)
- Emergency Eyewashes and Showers
- Compressed Gas Cylinder and Cryogenic Safety

LSU Health NEW ORLEANS

Background and Purpose

- This training is required every two years for all personnel who work in laboratories. It provides laboratory safety fundamentals, and shall be augmented by lab-specific training that addresses hazards specific to the lab. Note that the Biological Safety Training module has been eliminated and that module has been incorporated into this training.

- Principal Investigators should contact the Laboratory Safety Officer, Taylor Kriete, at tkriet@lsuhsc.edu, shortly after reporting onboard so that an initial laboratory safety inspection can be completed using the checklist contained in the Laboratory Inspection Program.

- When a Principal Investigator leaves the Health Sciences Center, follow the Lab Closeout Policy so that “vacated research space is thoroughly cleaned and prepared for reassignment to another investigator or unit.”

Housekeeping
Fire Safety

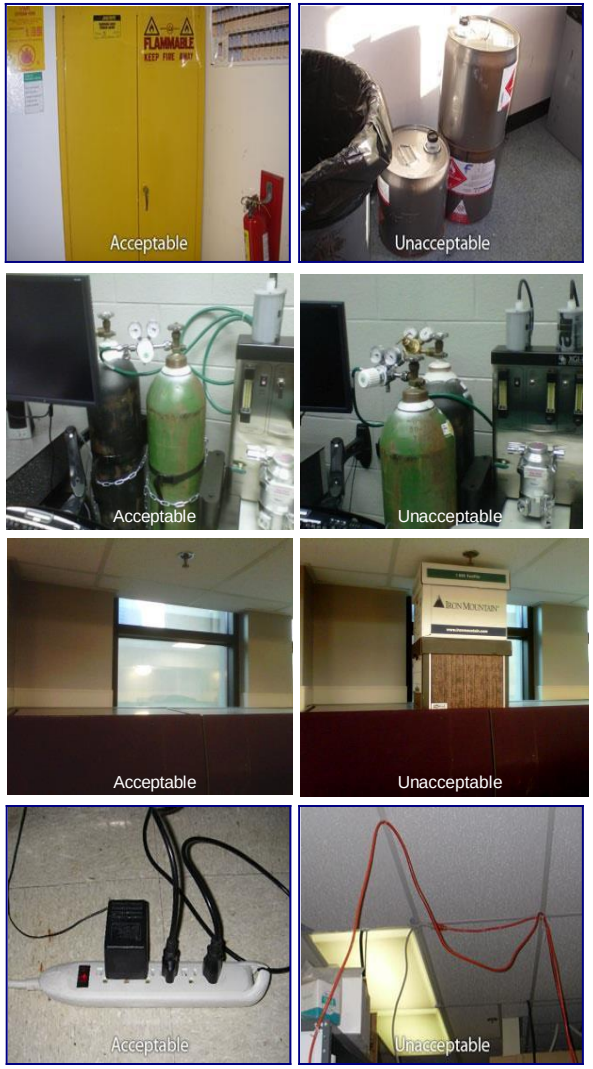
Housekeeping

- Eating or drinking is not allowed in laboratories that contain either chemical, biological or radioactive sources.
- Dispose of trash when it is generated in appropriate containers. Prevent accumulation of trash within the laboratory.
- Do not place empty bottles and other trip hazards in a walkway.
- Keep chemicals and glassware away from the edge of counters.
- Immediately clean up all spills.

Poor Housekeeping

Fire Safety

- Know the location of the nearest fire extinguisher. Ensure you have the equipment to address any special chemicals (e.g., pyrophoric or water-reactive chemicals).
- Flammable cabinet required if > 10 gals of any flammable present.
- Secure compressed gas cylinders.
- Maintain at least 18 inches of clearance between fire sprinkler heads and any other material.
- Ensure electrical wiring is free of cuts and not fraying.
- In the event of an alarm, secure any procedures that left unattended will pose a hazard and evacuate.



Chemical Safety

Hazard Communication

- The Hazardous Communications Program ensures that the hazards of all chemicals used at LSUHSC are evaluated and that information concerning their hazards is transmitted to all applicable personnel.

- A key element of the program is the Safety Data Sheet (SDS), which are used to familiarize the user with a chemical's:
  - Chemical and physical properties.
  - Toxicity and health effects.
  - Compatibility, safe handling and storage.
  - Spill and fire response.

- SDSs must be readily available, either in hard copy or on a computer hard drive.

Chemical Ordering, Storage, and Inventory

- When ordering chemicals, order only what is needed; a six month supply is plenty.

- In chemical storage areas:
  - Maintain chemical spill kits and fire extinguishers.
  - Provide adequate shelving; don't store on the floor.
  - Store quantities of 10 gallons or more of flammable materials in an approved Underwriters Laboratory (UL) or Factory Mutual approved flammable storage cabinet.
  - If cold storage is required for flammables, use a refrigerator that is UL approved and rated for flammable storage.

- Maintain an inventory of all chemicals per the Chemical Procurement, Inventory and Security Policy.

Chemical Incompatibilities

- To avoid interaction between incompatible chemicals, all chemicals should be separated into compatible hazard groups, then placed alphabetically within each group.
- Since many chemicals present multiple hazards, consult the SDS to determine the "primary" hazard class of a chemical.
- For more information on chemical storage, see Chemical Ordering and Storage Procedures.

Peroxide Forming Chemicals

A wide variety of organic compounds can spontaneously form peroxides on exposure to air. Peroxides are sensitive to heat, friction, and shock.

- Label containers with receiving, opening and disposal dates.
- Store away from heat sources and sunlight.
- Do not attempt to open a container of a peroxide forming compound if there are whitish crystals around the cap and/or in the bottle because it may be potentially explosive.
- For additional information on peroxide forming chemicals see the Standard Operating Procedure for Safe Use and Handling of Peroxide Forming Chemicals.

Engineering Controls – Fume Hoods

Chemical fume hoods are an important tool used to minimize exposure.

- Work with volatile chemicals in fume hoods whenever feasible.
- Hoods are not a place to store chemicals or equipment.
- Hoods are not designed to collect particulates and are not HEPA filtered. They are not to be used with infectious or potentially infectious materials.
- If your hood is not functioning properly, submit an on-line service request to have it repaired.

Fume Hoods (cont.)

Prior to Using a Fume Hood

- Fume hoods are inspected and certified annually by EH&S. Check the hood certification sticker on the front of the hood to ensure that the air flow is between 80 and 150 feet per minute when tested.
- Check visual alarms to ensure that the hood is functioning properly.
- Hold a Kimwipe or tissue at the face of the hood to ensure air is flowing into the hood when the fume hood is turned on.
- Check baffles to be sure slots are open and unobstructed by equipment or containers.
- Ensure that all chemicals and equipment are at least six inches behind the sash.
- Decrease turbulence by opening/closing sash slowly; avoid swift movements inside/outside of the hood.
- Keep sash as low as possible (closed when not in use).
- See the Standard Operating Procedures for the Safe Operation of Fume Hoods for more information.

Chemical Waste Disposal

- No chemical waste shall be discarded via the sink to the sanitary sewer system.
- When collecting waste, compatible waste streams may be collected in one container; ***never mix incompatible wastes.***
- Chemical disposal containers:
  - Use containers that are in good condition and are compatible with waste storage type.
  - Always keep the container closed during storage; never leave a waste container open with a funnel in it.
  - Use containers with capacities of one gallon or less and never over fill them.
- Label all waste containers with:
  - The words “Hazardous Waste”.
  - Principle chemical constituents.
  - Date that waste was first placed in the container.

Chemical Waste Disposal (cont.)

- To dispose of batteries:
  - Place in individual plastic bags or cardboard boxes for collection.
  - Cover terminals with tape if not placed in individual bags or boxes.

- For disposal of mercury containing equipment, place all equipment containing elemental mercury in a plastic bag and seal.

- Waste Pick-up:
  - Submit an on-line service request.
    for every hazardous chemical waste pick-up.
  - For additional information review the
    Chemical Waste Management Procedures

Chemical Spill Response

- Regardless of volume spilled, do not attempt to clean up a spill if the chemical is unknown or if known chemical is extremely toxic. If you are unable to safely clean the spill on your own, contact University Police at 568-8999. University Police will then notify Environmental Health and Safety to respond.

- See the Chemical Spill Response Policy and Procedures for more information.

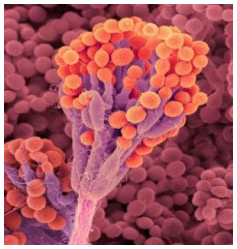
LSU Health NEW ORLEANS

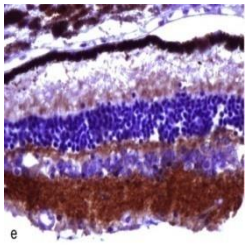
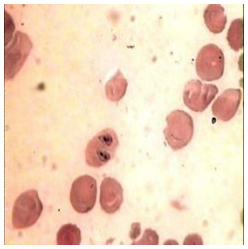
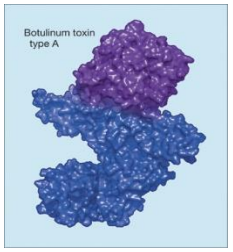
Biological Safety

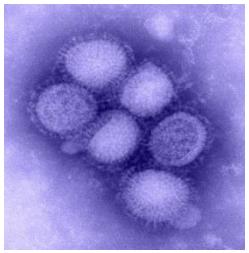
Biohazards and Exposure

- Biosafety is the combination of good laboratory practices, procedures, facilities, and safety equipment to protect human health and prevent release of biological materials into the environment.
- A biohazard is an agent of biological origin that has the capacity to produce deleterious effects in humans, such as microorganisms, and toxins and allergens derived from those microorganisms or higher plants and animals.
- Examples include:

Bacteria Fungi Parasites Prions Rickettsials Toxins Viruses



- Exposures are typically the result of needle sticks; accidental exposure to eyes, mouth and mucous membranes; bioaerosols; and spills.

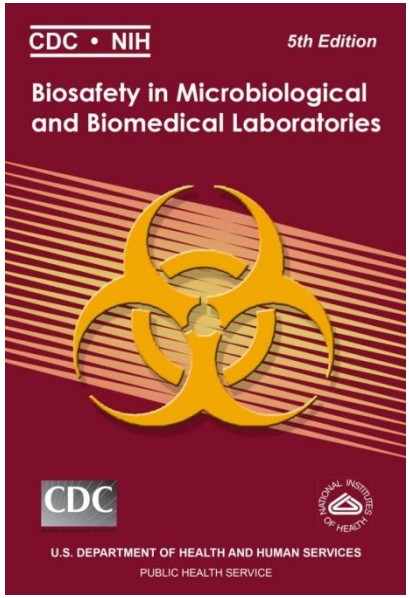
Biological Materials Inventory and Storage

- Maintain an inventory of all biological materials per the Biological Materials Inventory and Control Policy.

- Personnel who ship biological materials must complete the Shipping Biological Materials Training or equivalent dangerous goods training every two years.

Risk Assessment

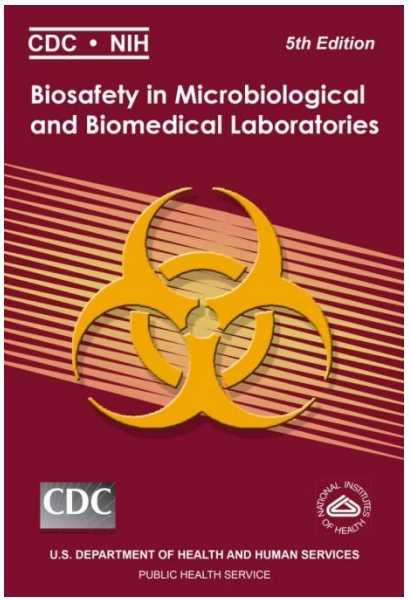
- Work practices, use of containment equipment, PPE, training, etc. should be guided by a thorough risk assessment (RA).
- A risk assessment ensures protection of personnel, the environment, the community and the integrity of *your* experiments.
- PIs are responsible for conducting the RA and should include the RA in lab-specific training and biosafety manuals.
- Risk assessment guidance can be found in the CDC *BMBL 5th Ed.*



Risk Assessment Process

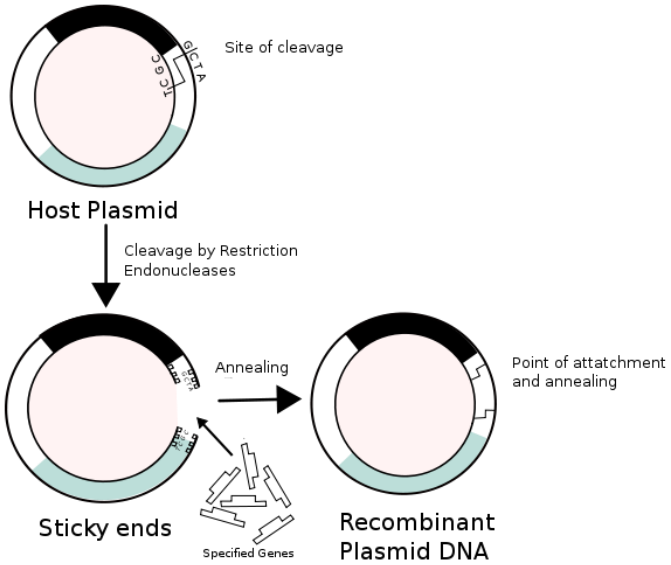
- RAs are comprised of four steps:
  1. Identification of health hazard
  2. Quantification of the hazard
  3. Exposure assessment
  4. The probability of disease

- And should include consideration of:
  - Virulence
  - Pathogenicity
  - Infectious dose
  - Environmental stability
  - Route of spread
  - Communicability
  - Operations and manipulation
  - Quantity and availability of vaccine or treatment



rDNA

- All rDNA used on campus is considered a biohazard. Experiments involving rDNA require specific biological barriers.
- Barriers should limit (i) the infectivity of a vector or vehicle – plasmid or virus – for specific hosts, (ii) its dissemination and survival in the environment
- Newer-generation vectors are designed to decrease the probability of dissemination of rDNA outside of the lab and are preferred.
- rDNA should be a part of the comprehensive risk assessment described in IBC documentation for your lab, taking into account source, vector, polypeptide or gene product, etc.



LSU Health NEW ORLEANS

Biological Safety
Biosafety Levels (BSLs)
Work Practices

Biosafety Level (BSL)

- BSL determination should be informed by risk assessment

A/BSL-1	**A/BSL-2**	**A/BSL-3**	**A/BSL-4**
Low individual risk (non-infectious to healthy adults)	**Moderate individual risk** (Not generally severe, treatment usually available)	**High individual risk** (Treatment may or may not be available)	**Severe individual risk** (Treatment often not available)
Low risk to community	**Low risk to community**	**Low risk to community**	**High risk to community**

Examples

- *E. Coli* lab strains *(e.g., DH5α, K12)*
- Mice
- Rats
- Rabbits

- Human cells, fluids, tissues
- NHP cells, fluids, tissues
- Lentiviral vectors
- Rhesus Macaques
- Toxins with an LD50 >100 ng/mg

Animals infected with BSL2 agents

- *M. tuberculosis*
- West Nile virus
- Yellow fever virus
- Monkeypox virus

Animals infected with BSL3 agents

- Ebola virus
- Lassa virus
- Marburg virus

Animals infected with BSL4 agents

Biosafety Level (BSL)

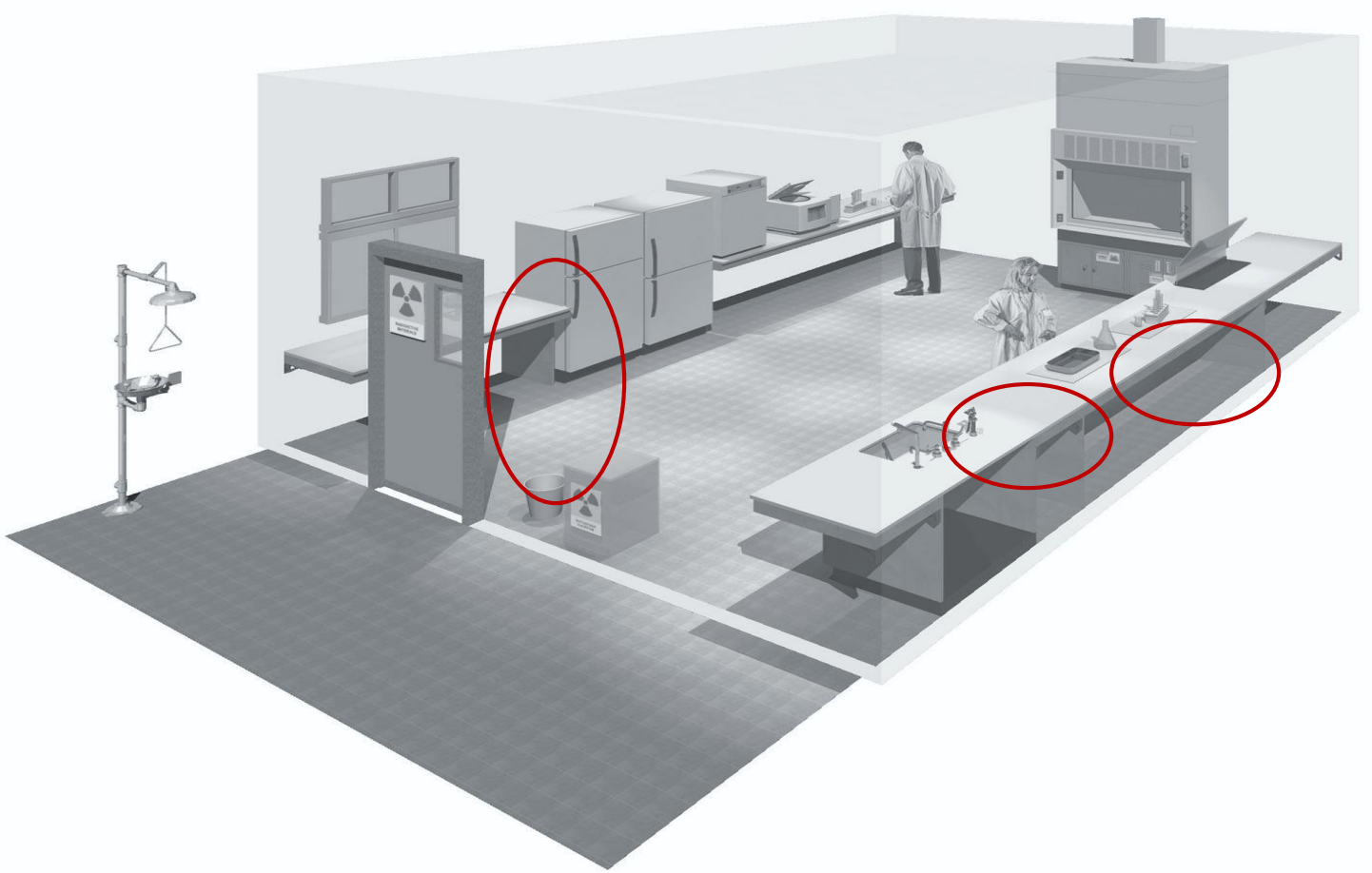
- PPE recommendation and guidance are available for each BSL

A/BSL-1	A/BSL-2	A/BSL-3	A/BSL-4
• Lab coats, gowns, etc. • Eye protection • Latex or nitrile gloves	• Lab coats, gowns, etc. • Eye protection • Latex or nitrile gloves -Change when contaminated -Double glove when necessary -Remove gloves and wash hands after working -Do not re-use gloves	• All manipulations performed inside a BSC • Full protective clothing that must not leave the lab • Eye protection • Latex or nitrile gloves • BSL-3 work practices • Appropriate respiratory protection	• All PPE indicated up to and including BSL-3 • Positive-pressure suit • Special facility engineering features

- A risk assessment pertinent to *your* lab and *your* work should yield PPE and work practice directives specific to *your* workplace

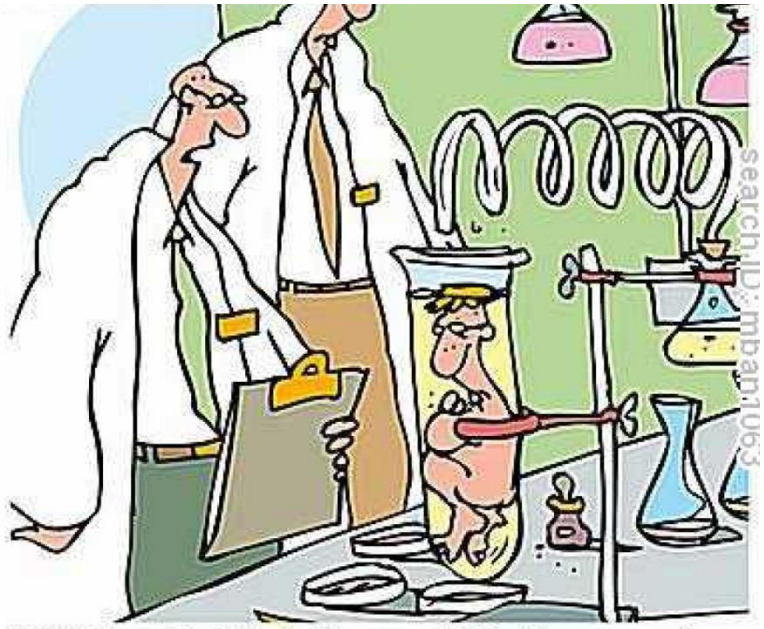
BSL-1 Facility Design

- Laboratory has doors to limit traffic.
- Hand washing sink is available.
- Work surfaces are easy to disinfect.



BSL-1 Work Practices

- Limit access when working.
- No eating, drinking, applying cosmetics or handling contact lenses.
- No mouth pipetting.
- Gloves must be worn and lab coats and protective eyewear are recommended.
- Minimize splashes and creation of aerosols.
- Disinfect waste and work surfaces.
- Biological waste should be placed in a biohazard disposal box, labeled, and placed outside for pickup when ¾ full.

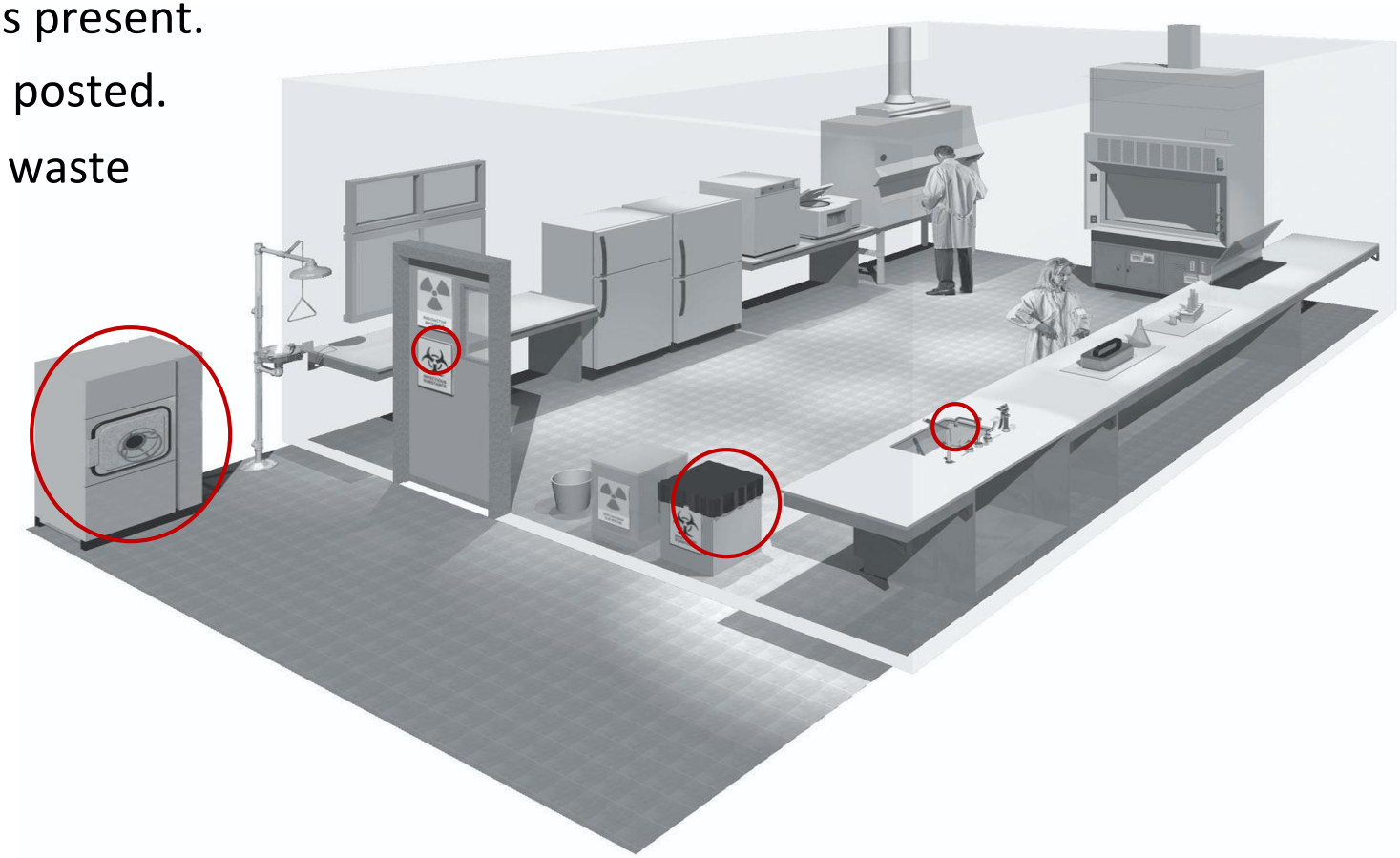


"Well, it certainly looks like your DNA. How many times have I told you to wear gloves before touching anything?"

BSL-2 Facility Design

All BSL-1 requirements, plus:

- Autoclave is available.
- Eyewash is present.
- Signage is posted.
- Biological waste stream is separate.



BSL-2 Work Practices

All BSL-1 practices, plus:

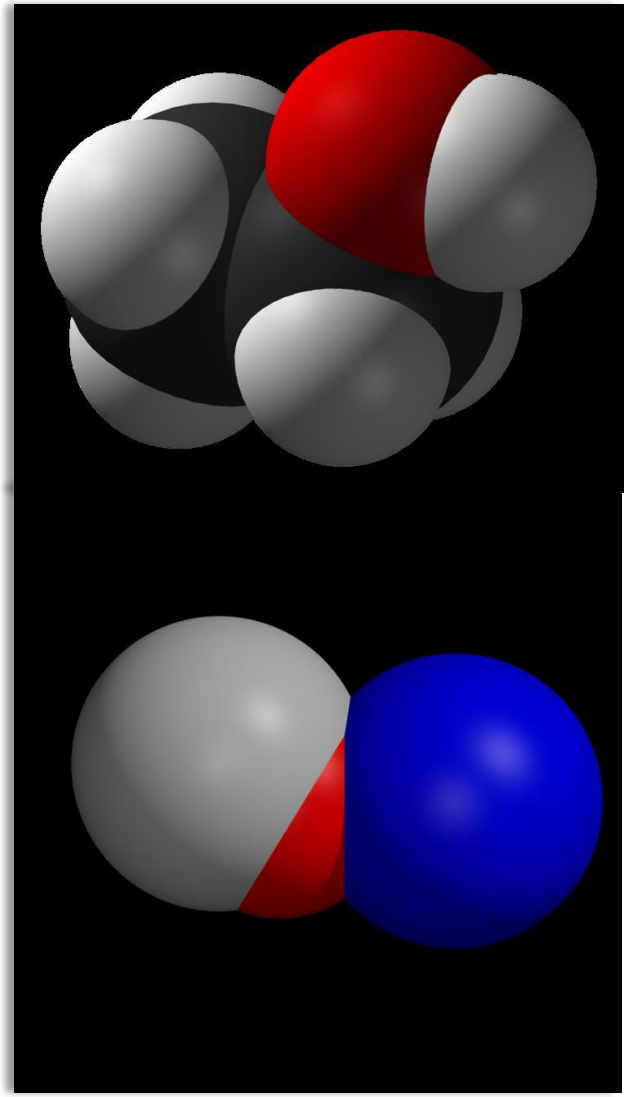
- A supervisor must limit access to those who are trained and approved.
- Policy for handling sharps must be implemented.
- Laboratory equipment must be routinely decontaminated.
- Protective lab coats or disposable gowns *must* be worn.
- Laboratory-specific Biosafety manual must be available in the lab.

Hand Washing

- All laboratories are required to have a sink available for hand washing.
- Wash hands for 15 seconds using warm water and mild – preferably liquid – soap.
- Rinse with warm running water.
- Dry with disposable paper towel.
- Alcohol-based hand sanitizers are an alternative to hand washing.
- Sanitizers are effective against common clinical microbes, but have not been tested against laboratory pathogens.
- Hand washing is preferred over hand sanitizers.

Disinfection

- Disinfection is the process of reducing a contaminant load
- Can be accomplished in the laboratory using a 70% solution of ethanol (EtOH) or a 10% solution of bleach (sodium hypochlorite)
- All works surfaces and materials should be disinfected before and after use
- SOPs for routine decontamination are available on the Biological Safety page of the EH&S website

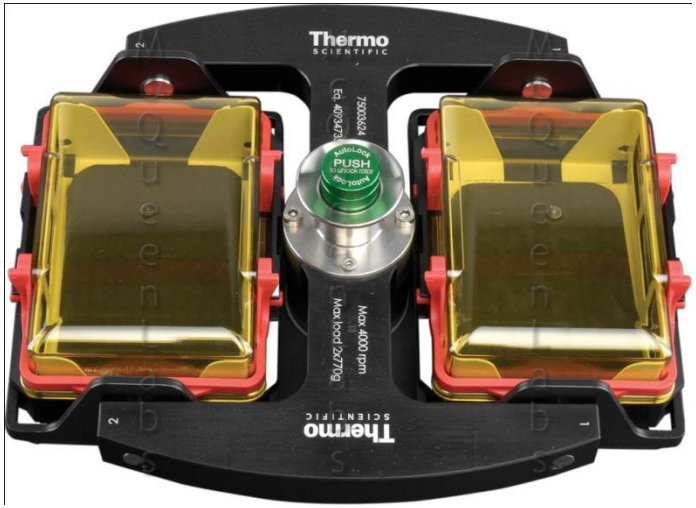


Autoclave Decontamination

- Place items in a secondary container made of stainless steel or autoclaveable plastic.
- Most pathogens and recombinant molecules are sensitive to temperatures above 121ºC for after 20 or more minutes.
- Larger loads require more time and should be arranged in a way that allows for steam penetration (i.e., not too densely packed).
- Do not cap vessels or add excessive liquid to the load.
- Use caution when opening autoclave at the end of the cycle – steam is usually still in the chamber.
- See Autoclave SOP for additional information.

Safe Handling of Liquids

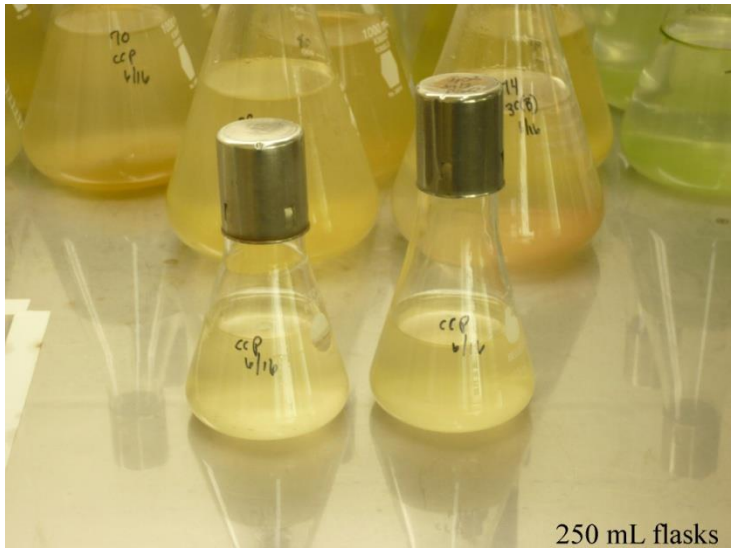
- Liquid biohazard materials (cultures, blood or body fluids must be placed in a container with a lid to prevent leaks and spills during collection, handling, processing, storage, transport or shipping).



Safe Handling of Liquids (cont.)

- Aqueous biological materials such as blood, cell cultures or microbial cultures must be either:
  - Decontaminated with bleach by adding 1 part bleach to each 9 parts liquid waste. Let stand for at least 60 minutes.

  OR
  - Decontaminate by autoclaving on liquid cycle.
  - *Do not* autoclave bleach-treated liquid waste.



Biological Safety Equipment

Biological Safety Cabinets

Biological Safety Cabinets (BSC) are an important tool used to control bioaerosols and minimize exposure.

- BSCs are designed to protect the product, worker, and environment. Laminar flow hoods only protect the product.
- BSCs remove particulates, not chemical vapors.
- All biosafety cabinets must be certified annually, prior to initial usage, and after being moved.
- At BSL2 and above, *any* procedure that *may* produce aerosols *must* be performed inside of a Biological Safety
- Contact EH&S before purchasing a new cabinet for help in selecting the appropriate cabinet.

Biological Safety Cabinets (cont.)

- Use High Efficiency Particulate Air (HEPA) filters.
- *Do not* protect against vapor or fumes, which may damage HEPA filters.

  Class I: Inward airflow protects personnel.

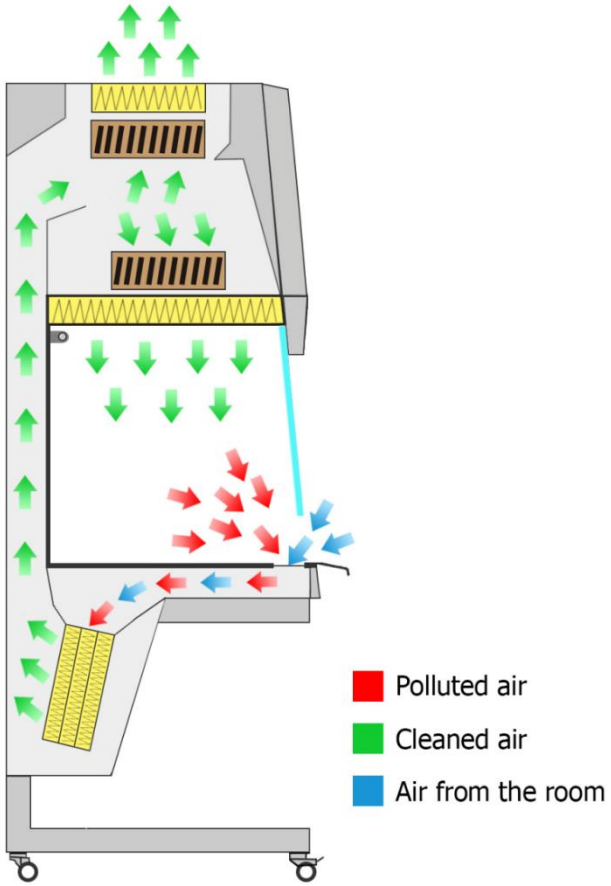
  Exhausts to outside.

  Class II:

  Four different types.

  Protects personnel, materials and environment with directional airflow and multiple HEPA filters, as pictured.

  Class III: Both inlet and exhaust air are HEPA filtered.

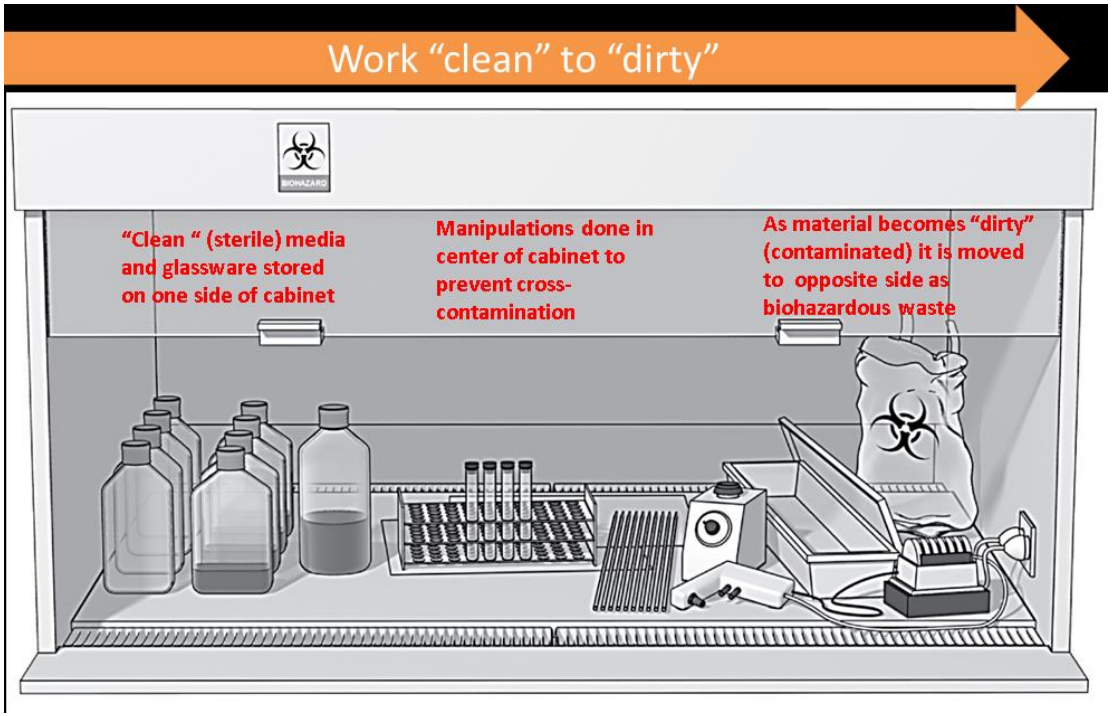


Safe Operations of BSCs – Before Use

- Disinfect cabinets before and after each use with 70% ethanol or 10% bleach solution.
- After disinfecting the cabinet, load supplies and allow the cabinet to run for 10-15 minutes before beginning work.
- Supplies should include a small autoclave bag, sharps container and beaker with disinfectant for liquid waste.
- Your BSC should have a current certification label. Contact EHS if certification or maintenance is needed.

Safe Operations of BSCs – During Use

- Check inward airflow by holding a piece of tissue near the opened sash.
- Segregate clean and dirty materials to avoid contamination.
- Place materials 4” or more inside cabinet to avoid disrupting airflow.
- When reaching into the cabinet to work, avoid abrupt or excessive movements to maintain airflow.



Safe Handling of BSCs – After Use

- When finished working, discard solid and liquid waste and sharps in the appropriate manner.
- Disinfect the work surface.
- Lower the sash.
- Turn on UV light.

Using BSCs with Radiation or Chemicals

Working with Radiation

- Radiolabeling of biological samples must be done inside a BSC.
- The BSC must be labeled with a Radiation Warning label.
- Proper shielding must be in place inside the BSC.

Working with Chemicals

- Some BSCs allow for work with *non-volatile chemicals* – some have restrictions on the quantity to be used.
- Check the indications for your particular class and type of BSC.

- More information can be found at the Standard Operating Procedure for the Safe Operation of Biological Safety Cabinets and NIH YouTube video.

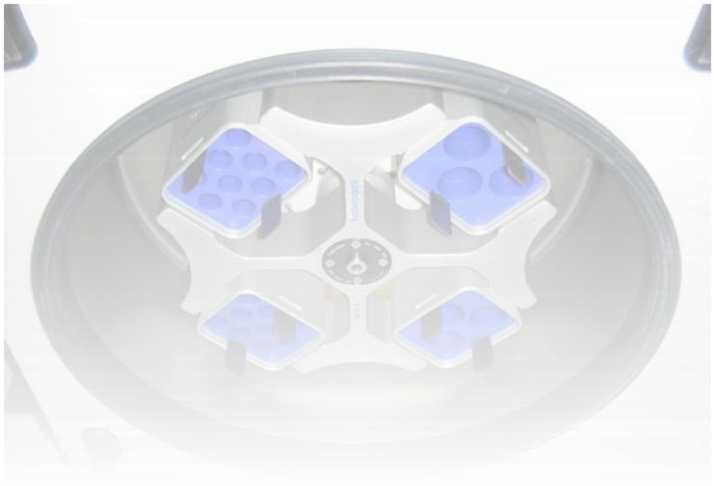
Laminar Flow Hoods

Laminar Flow Hood

- A laminar flow hood is *not a BSC* and *does not* provide personnel protection.
- Typically used for nucleic acid manipulation or other procedures that are very sensitive to contamination, but that do not pose a risk to personnel.
- Air flows out toward the user.
- Not to be used for work with infectious or potentially infectious materials.

Safe Operation of Centrifuges

- Check tubes for cracks, leaks or chips.
- Use matching sets of tubes and buckets to ensure that the centrifuge is properly balanced.
- Check that tubes and cups are sealed and that the rotor is locked and buckets are properly seated.
- Close lid firmly.
- When the cycle is finished, allow the rotor to come to a complete stop before opening lid.



Biological Safety
Waste Disposal, Spill Response and Biosecurity

Biological Waste Definition

Biohazardous waste includes the following general categories:

- Cultures and stocks.
- Pathological waste.
- Human and animal blood and blood products.
- Used and unused sharps (e.g., needles, syringes, pipettes, scalpels, broken glass, slides, cover slips).
- Animal waste (e.g., carcasses and bedding).
- Isolation wastes. Any other refuse, which has been mingled with any of the waste(s) listed above.

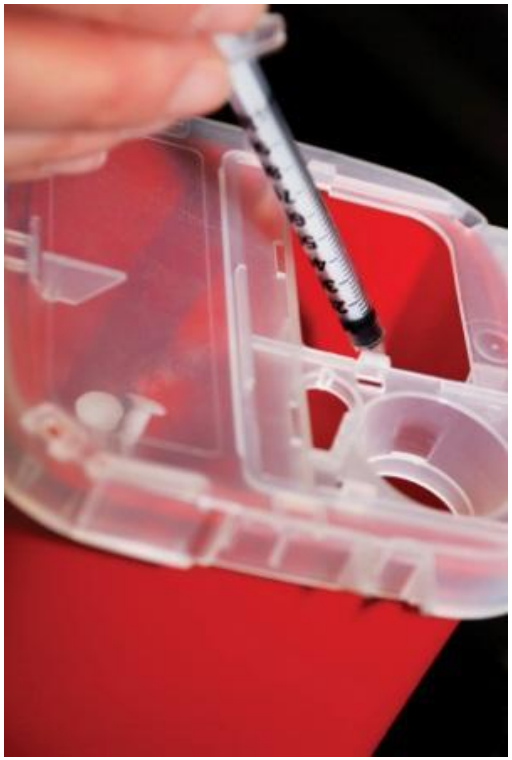
Never place chemicals, chemical bottles, radioactive materials, or other trash in biowaste containers.

Glass Disposal

- Uncontaminated or decontaminated glass may be disposed of in a designated, labeled cardboard box.
- Box should be sturdy and in good condition.
- Take care not to overload the box – it should be kept to a reasonable weight, approximately 25 lbs.

Sharps Disposal

- Used disposable needles *must not* be bent, altered, broken, recapped, removed from disposable syringes, or otherwise modified.
- Always dispose of contaminated sharps in an approved, puncture-resistant sharps container.
- Dispose of container when it is ¾ full by sealing the container and placing in a biological waste box.
- See the Standard Operating Procedure for the Safe Handling of Sharps for more information.



Bioboxes

- To be used for
  - all items contaminated with human or animal blood, fluid or tissue.
  - stocks, cultures or waste from infectious materials or microorganisms.
  - All materials that may be contaminated with recombinant molecules.
- Do not place sharps directly into the biological waste box. Place sealed sharps containers in a biological waste box.
- When box is ¾ full or reaches 25 lbs. close and tie liner, securely close lid, label with PI name and room number and place in hallway for pickup.

Spills and Potential Exposures

- Report all incidents involving potential exposure to rDNA to the Biological Safety Officer immediately. If the spill occurs after hours notify University Police. Depending on the type of incident and the level of exposure, notification of the NIH may be required within 24 hours of the incident.

- Response to spills depends on:
  - Agent identity and associated risks.
  - Agent's biosafety level.
  - Amount and location of the spill.

- If you need assistance to safely clean the spill then contact University Police at 568-8999. University Police will then contact Environmental Health and Safety to respond. See the Biological and rDNA Spill Response Procedures for more information.

Biosecurity

- In order to ensure the security of potentially harmful biological materials:
  - Control access to areas where biological agents and toxins are stored.
  - Know who is in your work area.
  - Know what materials are being brought into and taken out of your laboratory.
  - Have a protocol in place for reporting incidents or suspicious activities or people.

LSU Health NEW ORLEANS

Radiation and Laser Safety

Approval for Use of Radiation Sources

- Prior to beginning work with any radiation source, the PI must submit the Radiochemical Use Application Form to the Radiation Safety Committee for approval. Furthermore, personnel must complete Radiation Safety Training available from the Radiation Safety Officer (RSO).

- Therefore, if you intend on using radiation sources, please contact the RSO, James Davis, at jdavis3@lsuhsc.edu or 314-5989 to schedule training.

- For additional information see the LSUHSC Radiation Safety Committee Charter.

LSU Health NEW ORLEANS

Approval for Use of Lasers

- Prior to beginning work with any category 3b or 4 laser, lasers must be registered with the Radiation Safety Office.
- Additionally, "on-line" Laser Safety training must be completed.
- Therefore, if you intend on using class 3b or 4 lasers, please contact the RSO, James Davis, at jdavis3@lsuhsc.edu or 314-5989.
- For additional information see the LSUHSC Laser Safety webpage.

LSU Health NEW ORLEANS

Personal Protective Equipment

LSU Health NEW ORLEANS

Personal Protective Equipment

- Do not wear shorts or any clothing that exposes any skin other than arms or face.
- Do wear the appropriate Personal Protective Equipment (PPE) for the situation:
  - Gloves
  - Splash Goggles / Face Shields
  - Lab Coats
  - Respirators
- Provide proper storage of all PPE.
- See the Laboratory Attire and PPE and Personal Protective Equipment Policy web pages for more information.

Lab Coats and Gowns

- Lab coats or disposable gowns should be worn over street clothes any time you handle hazardous materials.
- The type of covering necessary and the frequency of changing is specific to your work and should be indicated in lab-specific training.
- Lab coats and gowns *must never be worn outside of the work area*
- See the Lab Coat Use, Selection and Cleaning web page for more information.

Eye and Face Protection

- Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials.
- People who wear contact lenses should also wear eye protection.
- At BSL2 and above, eye and face protection must be used for anticipated splashes and sprays of infectious materials when the microorganism is handled outside of a biosafety cabinet or other containment device.

Respiratory Protection

- Respirators must be worn when there is potential exposure to contaminants such as bioaerosols, dry chemicals and airborne particulates.
- Surgical masks do not provide respiratory protection.
- If you need an N95, full- or half-face N100, PAPR, or any other kind of fitted respirator, contact EH&S for a fit test.
- Fit testing requires a medical evaluation.
- See Respiratory Protection Program for more information.

Gloves

- The type of glove (material) used is dependent upon the chemical being handled.
- When using corrosive or toxic chemicals the gloves should be long enough to protect the forearm.
- Long gloves can be cuffed at the bottom to help prevent chemicals from running down the arm.
- Proper glove selection information can be accessed online at Ansell 8th Edition Glove Guide.

Gloves (cont.)

- Latex or nitrile gloves should be used for all handling of biological materials.
- Double gloves may be needed in some circumstances in order to avoid exposure or contamination.
- The type of gloves necessary and the frequency of changing is specific to your work and should be indicated in lab-specific training.
- Gloves must never be worn outside of the work area.

Goggles and Face Shields

- Splash goggles must be worn whenever lab workers are using liquid chemicals that could injure the eyes, including heated liquids.

- Face shields must be worn in conjunction with splash goggles if lab workers are using chemicals that could splash and corrode or burn the face, or when using toxic chemicals that could be splashed and absorbed through the skin.

PPE Storage

Personal protective equipment should be stored in a manner that protects the equipment from:

- Dust
- Sunlight
- Excess moisture
- Extreme temperatures
- Deformation
- Chemical degradation
- For more information see the PPE Policy.

Improper Storage

LSU Health NEW ORLEANS

Emergency Eyewashes and Showers
Compressed Gasses and Cryogenics

Emergency Eyewashes and Showers

- Eyewash and shower stations must be accessible to lab personnel that are using chemicals that could injure the eyes/skin.
- If equipment or supplies are located too close to this safety equipment, you may not be able to freely access the shower or eyewash. Furthermore, the equipment could become damaged during use or testing. For more information see the Emergency Eyewash and Shower Policy.

Compressed Gas Cylinders

- Cylinders could act as a projectile if dropped and damaged.
- Secure cylinders to stationary objects (like a wall).
- Remove regulators and install valve protection caps when cylinders are not in use.
- If cylinder not properly secured, create an on-line service request. See the Compressed Gas Cylinder Policy for more information.

"Not secured"

"Properly secured"

Cryogenic Liquids

- Liquid nitrogen (LN_2) is extremely cold (-320F).
- Displaces oxygen and expands rapidly.
- Protect eyes and skin with goggles, gloves, shoes.

- Safe handling tips for (LN_2)
  - Do not allow to touch bare skin.
  - Do not seal containers.
  - Transfer liquid with care.
  - Transport containers with care.
  - Handle containers with care.

- For more information see the Cryogenic Liquid Policy and the Liquid Nitrogen Supply and Safety Plan.

More Information and Points of Contact

- Further information on these and all LSUHSC Environmental Health and Safety programs can be found at the EH&S web site.
- If you have questions on this training please contact the following:
  - Taylor Kriete, Biological and Chemical Safety Officer, at tkriet@lsuhsc.edu
  - James Davis, Radiation Safety Officer, at jdavis3@lsuhsc.edu.