NIA IRP SUMMER INTERN LABORATORY SAFETY MANUAL 2008

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RESPONSIBILITY

As a summer intern you will work under the supervision of your mentor and various other investigators. However,

YOU ARE ULTIMATELY RESPONSIBLE FOR YOUR SAFETY

While your duties will vary according to your interest and NIA IRP Branch assignment, you must follow all safety procedures developed by the NIH and NIA IRP. All of the policies and procedures are mandated by the federal government through the National Institutes of Health (NIH), the Occupational Safety and Health Administration (OSHA), the Environmental Protection Agency (EPA) and various other agencies.

A working understanding of laboratory safety is integral to any scientific discipline. Aside from injury, loss of resources, and recovery time, laboratory incidents have legal ramifications. An organization can be fined for safety violations even when there are no accidents. Safety knowledge is a skill set that employers expect scientists to have.

Common Causes of Lab Accidents

- Lack of working understanding of hazards
- Improper or unintended use of equipment or reagents
- Inexperienced scientist
- Distractions, lack/loss of attention to task
- Broken, damaged glassware or equipment

You are required to report all work-related accidents, injuries and illnesses to your supervisor, mentor, and the Safety Office. You have the right to know the dangers of the material and equipment used. In addition you are required to wear the Personal Protective Equipment (PPE) provided for your protection. While working in the lab you are expected to dress and behave appropriately. Paying attention to the task you are performing is critical in preventing accidents in the laboratory. Try to do one thing at a time, and do it well. Anticipate and minimize distractions such as phone calls, radios, talking while performing your experiments.

YOU WILL BE TESTED ON THE MATERIAL IN THIS MANUAL

APPROPRIATE DRESS AND BEHAVIOR

APPROPRIATE DRESS HELPS TO ENSURE YOUR SAFETY

- Do not wear shorts, sandals, open toe shoes or shoes made of woven material. Sturdy clothes and shoes that cover the entire foot are necessary for protection against corrosive materials and mishaps with equipment.

- Protect your skin. Wearing bare midriffs or low cut pants may result in severe scaring if you or a coworker has an accidental spill or splash.
- Be aware of dangling jewelry, loose or baggy clothing, or long hair that might get caught in equipment.
- If dressed inappropriately, you may be asked to leave the lab and return in proper attire.

Professional standards of personal behavior are expected in any laboratory.

- Avoid distracting or startling other workers.
- Conduct yourself in a responsible manner at all times in the laboratory. Do not engage in practical jokes or horseplay.
- Do not sit on the laboratory benches or tables.
- Do not allow visitors in laboratories where hazardous materials are stored or are in use. Access to the labs should be limited to authorized personnel only.
- Do not eat, drink, chew gum or apply cosmetics in the laboratory.
- Contact lenses are not recommended for laboratory work since that may trap chemicals causing injury to the eye.

SAFETY TRAINING

Detailed safety and chemical handling procedures will be explained to you in a number of safety training classes. You and your mentor will receive a detailed training schedule based on you work assignment.

NIA IRP Baltimore On-site Orientation – FOR NEW EMPLOYEES ONLY
This information will be presented during the initial orientation. If you begin working before or after the orientation date a CD-ROM will be issued by the Safety Office. Upon completion, return the CD and you will be given a short quiz on the material.

Introduction to Laboratory Safety – CD-ROM or available online at http://www.ors.od.nih.gov/labsafety/ , REQUIRED FOR ANYONE WORKING IN A LABORATORY. Upon Completion please send a copy of your certificate of completion to the Safety Office in Room 1D20.

NIH Laboratory Safety - REQUIRED FOR ANYONE WORKING IN A LABORATORY

Working Safely with HIV and BBP - (ONLY IF YOU WORK WITH HUMAN SUBJECTS, HUMAN BLOOD AND BODY FLUIDS, OR HUMAN CELL LINES)

NIA IRP Annual On-site Safety Training –REQUIRED EVERY YEAR FOR RETURNING EMPLOYEES, INTERNS, STUDENTS, AND CONTRACTORS REGARDLESS OF JOB RESPONSIBILITIES

All new summer interns will be required to complete the first three courses. Depending upon your job responsibilities you may also be required to take "Working Safely with HIV and Other Bloodborne Pathogens. Returning summer interns are required to complete the annual safety refresher titled NIA IRP Annual On-site Safety Training. Additional training may be required if your responsibilities have changed.

GENERAL SAFETY RULES

- Become familiar with the locations and use of all safety equipment including eye wash stations, safety showers, spill control materials, fire blanket, fire extinguishers, fire alarms and fire exits.
- Keep the aisles clear.
- Do not work alone.
- Observe good housekeeping practices.
- Remove gloves carefully; thoroughly wash hands and forearms upon completion of work and before leaving the laboratory.
- Never pipet by mouth.
- Examine glassware before each use. Do not use dirty, chipped, broken or cracked glassware in any procedure.
- Do not prepare or store food or drink in any laboratory.
- Do not taste, touch or smell any reagents.
- Always wash your hands before leaving the laboratory. This will prevent contamination of your food or person.
- Wear laboratory coats and other protective clothing while performing laboratory activities. Do not wear this clothing outside of the laboratory.
- Wear appropriate gloves while handling infectious or toxic materials and animals.

WORKING WITH CHEMICALS

There are hazards associated with most chemicals. During your safety trainings at the NIA IRP, you will be given information on hazard identification, safety protocols and emergency procedures. Please remember even though you will be given training, personal protective equipment, and supervision, you are responsible for your safety. In addition to the general safety protocols listed above, there are additional safety measures needed when working with chemicals:

- Do not store chemicals near heat sources such as ovens or steam pipes. Also, do not store chemicals in direct sunlight.
- Ensure unimpeded access to safety showers and eyewash stations. Test flush eyewash stations weekly.
- Do not use lab benches as permanent storage for chemicals. In these locations, the chemicals can easily be knocked over, incompatible chemicals can be stored alongside one another, and the chemicals are unprotected in the event of a fire.
- Each chemical must have a proper designated storage location and be returned there after use.

- Inspect your chemicals routinely for any signs of deterioration and for the integrity of the label. State and federal law requires that **all** chemicals must be clearly labeled.
- Do not store any chemicals in glass containers on the floor.
- Do not use a Chemical Fume Hood (CFH) as a permanent storage location for chemicals, with the exception of particularly odorous chemicals that may require ventilation. The more containers, boxes, equipment, and other items that are stored in a CFH, the greater the likelihood of having chemical vapors being drawn back into the room.
- Chemicals that require refrigeration must be sealed with tight-fitting caps and kept in lab safe refrigerators. Lab safe refrigerators/freezers must be used for cold storage of flammables.
- Do not store chemicals above eye level. If the container breaks, the contents can fall onto your face and upper body.
- Use a chemical fume hood when opening, pouring, or handling hazardous chemicals.
- Transport laboratory chemicals using bottle carriers and suitable carts.
- The identification and disposal of unlabeled chemical containers is very expensive. Label all chemical containers in the laboratory with the following information:
    - The name of the chemical or stock solution
    - The date of preparation
    - Concentration
    - Your initials
- Do not dispose of chemicals down the drain or by evaporation.
- Properly collect, tag, and date waste. Keep chemical waste containers closed/sealed. Use drip pans under waste collection containers to prevent spills.

Flammable/Combustible Liquids

Flammable liquids are indeed the most common chemicals found in a laboratory. The primary hazard associated with flammable liquids is, of course, their ability to readily ignite and burn. One should note that it is the vapor of a flammable liquid, not the liquid itself, that ignites and causes a fire. The rate at which a liquid vaporizes is a function of its vapor pressure. Vapor pressure increases rapidly as the temperature is raised as does the evaporation rate. A reduced-pressure environment also accelerates the rate of evaporation. The flash point of a liquid is the lowest temperature at which a liquid gives off enough vapor to form a mixture that will burst into flame if given a source of ignition.

- Flammable liquids that are not in active use must be stored in safe containers inside fire resistant storage cabinets designed for flammables.
- Use flammables only in areas free of ignition sources.
- Do not heat flammables with an open flame. Instead, use steam baths, water baths, oil baths, hot air baths, sand baths or heating mantles.
- Never store flammable chemicals in a standard household refrigerator. There are several ignition sources located inside a standard refrigerator that can set off a fire or violent explosion. Flammables can only be stored cold in a lab safe or

explosion-proof refrigerator. Another alternative is to use an ice bath to chill the chemicals.

Oxidizers

Oxidizers or oxidizing agents present fire and explosion hazards on contact with combustible materials. Depending on the class, an oxidizing material may increase the burning rate of combustibles with which it comes in contact; cause the spontaneous ignition of combustibles with which it comes in contact; or undergo an explosive reaction when exposed to heat, shock, or friction. Oxidizers are generally corrosive.

- Store oxidizers away from flammables, organic compounds, and combustible materials.
- Strong oxidizing agents like chromic acid should be stored in glass or some other inert container, preferably unbreakable. Corks and rubber stoppers should not be used.
- Reaction vessels containing appreciable amounts of oxidizing material should never be heated in oil baths, but rather on a heating mantle or sand bath.

Corrosives

Corrosives are most commonly acids and alkalis, but many other materials can be severely damaging to living tissue. Corrosives can cause visible destruction or irreversible alterations at the site of contact. Inhalation of the vapor or mist can cause severe bronchial irritation. Corrosives are particularly damaging to the skin and eyes. Please be aware that certain substances considered non-corrosive in their natural dry state are corrosive when wet such as when in contact with moist skin or mucus membranes. An Examples of these materials are lithium chloride, halogen fluorides, and allyl iodide. Sulfuric acid is a very strong dehydrating agent and nitric acid is a strong oxidizing agent. Dehydrating agents can cause severe burns to the eyes due to their affinity for water.

- Always store acids separately from bases. Also, store acids in acid storage cabinets away from flammables since many acids are also strong oxidizers.
- Do not work with corrosives unless an emergency shower and continuous flow eyewash are available. Know the location and correct operation of these essential pieces of safety equipment.
- Add acid to water, but never add water to acid. This is to prevent splashing from the acid due to the generation of excessive heat as the two substances mix.
- Never store corrosives above eye level. Store on a low shelf or cabinet.
- It is a good practice to store corrosives in a tray or bucket to contain any leakage.

Water Reactive Materials

When water reactive materials come in contact with water, one or more of the following can occur: liberation of heat which may cause ignition of the chemical itself if it is flammable, or ignition of flammables that are stored nearby; release of a flammable, toxic, or strong oxidizing gas; release of metal oxide fumes; and formation of corrosive acids. Water reactive chemicals can be particularly hazardous to firefighting personnel responding to a fire in a lab, because water is the most commonly used fire extinguishing medium.

Peroxide-Forming Materials

Peroxides are very unstable and some chemicals that can form them are commonly used in laboratories. This makes peroxide-forming materials some of the most hazardous substances found in a lab. Peroxide-forming materials are chemicals that react with air, moisture, or impurities to form peroxides. The tendency to form peroxides by most of these materials is greatly increased by evaporation or distillation. Organic peroxides are extremely sensitive to shock, sparks, heat, friction, impact, and light. Many peroxides formed from materials used in laboratories are more shock sensitive than TNT. Just the friction from unscrewing the cap of a container of an ether that has peroxides in it can provide enough energy to cause a severe explosion.

Examples of peroxide-forming materials: diisopropyl ether, divinylacetylene sodium amide, potassium amide, dioxane, diethyl ether, tetrahydrofuran vinyl ethers, butadiene, vinylpyridine, acrylonitrile, styrene.

- Do not open the chemical container if peroxide formation is suspected. The act of opening the container could be sufficient to cause a severe explosion. If you notice crystal formation in the container or around the cap, do not attempt to open or move the container. Call the Safety Office or inform your mentor.
- Visually inspect liquid peroxide-forming materials for crystals or unusual viscosity before opening. Pay special attention to the area around the cap. Peroxides usually form upon evaporation, so they will most likely be formed on the threads under the cap.
- Date all peroxide forming materials with the date received, and the expected shelf life. Chemicals such as diisopropyl ether, divinyl acetylene, sodium amide, and vinylidene chloride should be discarded after three months.
- Store all peroxide-forming materials away from heat, sunlight, and sources of ignition. Sunlight accelerates the formation of peroxides.
- Secure the lids and caps on these containers to discourage the evaporation and concentration of these chemicals.
- Never store peroxide-forming materials in glass containers with screw cap lids or glass stoppers. Friction and grinding must be avoided. Also, never store these chemicals in a clear glass bottle where they would be exposed to light.

Compressed Gas Cylinders

Cylinders of compressed gases can pose a chemical as well as a physical hazard. If the valve were to break off a cylinder, the amount of force present could propel the cylinder through a brick wall. For example, a cylinder of compressed breathing air used by SCUBA divers has the explosive force of 1 1/2 pounds of TNT.

- Always use the appropriate regulator on a cylinder. If a regulator will not fit a cylinder's valve, replace the cylinder, not the regulator. Do not attempt to adapt or modify a regulator to fit a cylinder it was not designed for. Regulators are designed to fit only specific cylinder valves to avoid improper use.
- Inspect regulators, pressure relief devices, valves, cylinder connections, and hose lines frequently for damage.

- Never use a cylinder that cannot be positively identified. Color coding is not a reliable way of identifying a cylinder because the colors can vary from supplier to supplier.
- Do not use oil or grease on any cylinder component of an oxidizing gas because a fire or explosion can result.
- Never transfer gases from one cylinder to another. The gas may be incompatible with the residual gas remaining in the cylinder or may be incompatible with the cylinder material.
- Place all cylinders so that the main valve is always accessible.
- Close the main cylinder valve whenever the cylinder is not in use.
- Remove regulators from unused cylinders and always put the safety cap in place to protect the valve.
- Always secure cylinders, whether empty or full, to prevent them from falling over and damaging the valve (or falling on your foot). Secure cylinders by chaining or strapping them to a wall, lab bench, or other fixed support.
- To transport a cylinder, put on the safety cap and strap the cylinder to a hand truck in an upright position. Never roll a cylinder.
- Always clearly mark empty cylinders and store them separately.
- Be careful while handling compressed gas cylinders and never drop or strike a cylinder against anything.
- Use only wrenches or other tools supplied by the cylinder supplier to open a valve.
- Open cylinder valves slowly.

BIOLOGICAL HAZARDS

Working with bio-hazardous material requires special safety precautions. Viruses, bacteria and other microorganisms can enter the body through contact with non-intact skin or mucus membranes, by inhalation, ingestion and inoculation. Protection is provided by primary barriers such as biological safety cabinets, personal protective equipment and special procedures.

Standard Microbiological Practices

- Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.
- Eating, drinking, handling contact lenses, and applying cosmetics are not permitted in work areas where there is reasonable likelihood of exposure to potentially infectious materials.
- Persons who wear contact lenses in laboratories should also wear goggles or a face shield.
- Food is stored outside the work area in cabinets or refrigerators labeled and used for this purpose only.
- Mouth pipetting is prohibited; mechanical pipetting devices are used.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.

- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method.
- It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.
- Gloves should be worn if the skin on the hands is broken or if a rash exists.
- Protective eyewear should be worn for anticipated splashes of microorganisms or other hazardous materials to the face.
- Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices).
- All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
- Gloves are worn when handling infected animals and when hands may contact infectious materials, contaminated surfaces, or equipment. Wearing two pairs of gloves may be appropriate; if a spill or splatter occurs; the hand will be protected after the contaminated glove is removed.
- Gloves are disposed of when contaminated, removed when work with infectious materials is completed, and are not worn outside the laboratory.
- Disposable gloves are not washed or reused.

ENGINEERING CONTROLS

Engineering controls are the first line of defense against chemical and biological hazards. When working with certain hazardous chemicals a chemical fume hood (CFH) is used. When not in use, flammable and combustible materials are kept in a flammable safety cabinet (FSC). Protection from potentially infectious material is provided by a biological safety cabinet (BSC).

Chemical Fume Hood (CFH)

Work that involves hazards and noxious materials which are toxic, odoriferous, volatile or harmful shall be conducted within a laboratory hood. The primary purpose of a laboratory hood is to keep toxic or irritating vapors and fumes out of the general laboratory working area. A secondary purpose is to serve as a shield between the worker and equipment being used when there is the possibility of an explosive reaction. This is done by lowering the sash of the hood.

- Always assure the hood is operational before initiating an experiment.
- Do not use infectious material in a chemical fume hood.
- Only items necessary to perform the present experiment should be in the hood. The more equipment in the hood, the greater the air turbulence and the chance for gaseous escape into the lab.
- Substitute toxic chemicals with less hazardous materials whenever possible.

- Keep fume hood exhaust fans on at all times.
- Perform all work six inches inside the hood.
- Never place your head inside the hood.
- Keep the hood sash closed as much as possible at all times to ensure the optimum face velocity and to minimize energy usage.
- Keep lab doors closed to ensure negative room pressure to the corridor and proper air flow into the hood.
- Do not store chemicals in the hood.
- Keep the slots of the baffle free of obstruction.
- Do not use the hood as a waste disposal mechanism (e.g., for evaporation of chemicals).
- Avoid rapid movements in front of the hood including opening and closing the fume hood sash rapidly and swift arm and body movements in front of or inside the hood. These actions may increase turbulence and reduce the effectiveness of fume hood containment.
- Do not override or disable mechanical stops on the sash.

Biological Safety Cabinet (BSC)

A biological safety cabinet (BSC) works by pulling room air across the cabinet work surface and through a high efficiency particulate air (HEPA) filter. Part of the air is re-circulated within the BSC and part is returned to the room. Because of this room air return, do not use hazardous chemicals in a BSC.

- Minimize movement in and out of the BSC. Do not use a sweeping motion. Move arms in and out slowly, perpendicular to the face opening.
- As much as possible, reduce other activities in the room. Minimize rapid movement near the BSC as well as open/closing of the room door
- Materials or equipment placed inside the cabinet may disrupt airflow, resulting in turbulence, possible cross contamination, and/or breach of containment. Extra supplies should be stored outside the cabinet. Only materials and equipment required for the immediate work should be placed in the BSC.
- Avoid blocking the front or back grille. Do not place papers, pipetting devices, etc. on the grille. Do not rest arms on the front grille while working. This may draw room air directly into the cabinet. To prevent this, raise arms slightly above the grille surface.
- Allow the cabinet to stabilize and air sweep to occur. To remove particles in the cabinet, allow the blowers to run at least 15 minutes prior to beginning work. Delay manipulation of material for one minute after placing hands/arms in the BSC to air sweep the arms and hands of surface microbial contaminants.
- Work 4 - 6 inches inside the cabinet.
- An open flame creates air turbulence. Do not use a Bunsen burner. If absolutely necessary, a touch-plate microburner may be used (Touch-O-Matic).
- To reduce contamination, wipe containers and material with 70% EtOH prior to placing them in the cabinet.

- Work should flow from the clean to the "dirty" areas of the cabinet. Clean cultures on one side can be inoculated in the center and contaminated pipets placed in a container on the other side of the BSC.
- Decontamination of the cabinet interior should occur prior to and upon completion of work.
- Wipe down the interior of the glass, the work surface, cabinet sides and back. Take care not to wet the supply filter diffuser. A HEPA filter loses integrity when wet.

PERSONAL PROTECTIVE EQUIPMENT

Personal Protective equipment (PPE) protects only if it is worn and used properly. In prior years, a chemical splash has been the most common type of student injury. In those cases PPE was not being worn.

Protective Eyewear

- Goggles provide the best all around protection against chemical splashes, vapors, dusts, and mists.
- Goggles that have indirect vents or are non-vented provide the most protection.
- Standard safety glasses provide protection against impact.
- If using a laser, wear safety glasses or goggles which provide protection against the specific wavelength of that laser.
- Remember, prescription glasses do not provide adequate protection in a laboratory setting. Prescription safety glasses can be purchased from most opticians.
- Contact lenses should not be worn in a laboratory because they can trap contaminants behind them and reduce or eliminate the effectiveness of flushing with water from an eyewash. Contact lenses may also increase the amount of chemicals trapped on the surface of the eye and decrease removal of the chemical by tearing. If it is necessary to wear contact lenses in a lab, wear protective goggles at all times.

Protective Gloves

- Any glove can be permeated by chemicals. The rate at which this occurs depends on the composition of the glove, the chemicals present and their concentration, and the exposure time to the glove. If you are not certain which type of glove provides you with the protection you need, check with your mentor or contact the Safety Office at 410-558-8028.
- If direct chemical contact occurs, replace gloves regularly throughout the day.
- Remove gloves and wash hands regularly before using the telephone or opening doors to prevent the spread of contamination.
- Check gloves for cracks, tears, and holes.
- Butyl, neoprene, and nitrile gloves are resistant to most chemicals, e.g., alcohols, aldehydes, ketones, most inorganic acids, and most caustics.

- Disposable latex and vinyl gloves protect against some chemicals, most aqueous solutions, and microorganisms and reduce risk of product contamination. **Note:** There is increasing evidence that some people develop a serious allergic reaction to latex.
- Leather and some knit gloves will protect against cuts, abrasions, and scratches, but not against chemicals.
- Temperature-resistant gloves protect against cryogenic liquids, flames, and high temperatures.

Other Protective Equipment

- The primary purpose of a lab coat is to protect against splashes and spills. A lab coat should be nonflammable, where necessary, and should be easily removed.
- Face shields can protect against impact, dust, particulates, and chemical splashes for the face, eyes, and throat. However, chemical vapors and splashes can still travel under and around a face shield. Wear protective eyewear such as goggles underneath a face shield if there is a danger of aerosol formation or splashes that may come under the shield.

FIRE SAFETY

Most laboratories contain hazardous chemicals which may be flammable. It is important to follow the fire safety guidelines. These will be reviewed in greater detail during your safety training.

- Know the location of the fire alarm pull stations.
- Be familiar with two safe exits from your work place.
- In case of a fire, DO NOT USE THE ELEVATORS. Use the stairs to exit.
- Do not use a fire extinguisher unless you have completed Fire Extinguisher Training.
- If your clothes catch fire, do not run. Running will fan the flames. Drop to the ground and begin to roll to smother the flames. While rolling cover your nose and mouth with your hands. This will prevent inhalation of smoke and fire.