HISTOLOGY LABORATORY

WORKING WITH CHEMICALS

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WORKING WITH CHEMICALS

1 General safety policies

The following rules and procedures should be observed in all laboratory work with chemicals. Safety is your responsibility. You are working with materials that are extremely hazardous to you and your fellow workers. *Think safety—work safely.*

Do not take chances. Avoid emergencies by carefully planning ahead before starting a job. Know every step and possible hazards involved. Prepare for any emergency which may arise. Refer to specific chapters in this manual for more descriptive instructions and information.

1.1 Accidents and spills

1.1.1 Eye contact

Promptly flush eyes with water for at least 15 minutes and seek medical attention.

1.1.2 Ingestion

Encourage the victim to drink large amounts of water. Refer to the MSDS for special precautions and additional information.

1.1.3 Skin contact

Promptly flush the affected area with water and remove any contaminated clothing. If symptoms persist after washing, seek medical attention.

1.1.4 Cleanup

Promptly contain chemical spills and alert people in all parts of the facility including isolation areas. If the spill is small, clean it up using appropriate protective apparel and equipment. Remember to dispose of contaminated items as hazardous waste. For large spills or those containing extremely toxic or hazardous materials contact safety services personnel for assistance.

1.2 Eating, smoking, and cosmetics

Eating, smoking, drinking, gum chewing, or application of cosmetics are prohibited in areas where laboratory chemicals are present. Wash hands before conducting these activities. Avoid storing or handling food or beverages in storage areas, refrigerators, glassware, or utensils which are used for laboratory operations.

1.3 Equipment and glassware

Handle and store laboratory glassware with care to avoid damage. Damaged glassware should be discarded and not used. Use equipment only for its designed purpose. Read instructions before using laboratory equipment.

1.4 Exiting

Wash hands prior to leaving the laboratory.

1.5 Horseplay

Avoid practical jokes or other behavior that might confuse, startle or distract another worker.

1.6 Mouth suction

Do not use mouth suction for pipetting or starting a siphon. use pipet pumps.

1.7 Personal protective equipment

The MSDS will specify which protective equipment are to be used.

1.7.1 Eye protection

ANSl approved safety glasses or goggles are required in laboratories, chemical storage rooms and designated contaminated areas. They should be worn when weighing out chemicals, changing tissue processors, working with formaldehyde, and pouring solutions. Goggles and face shields are provided.

1.7.2 Gloves

Wear appropriate gloves when the potential for contact with toxic materials exists. Inspect the gloves before each

use and wash them before removal. Replace them frequently to avoid contaminating yourself and other objects such as door handles. The appropriate glove can be determined by consulting MSDSs or the compatibility lists from the glove manufacturers. Do not wear gloves out of the laboratory area. One hand must clean to open doors.

The appropriate gloves for this work area are

- nitrile when working with xylene, acetone and acids;
- latex for other materials associated with the laboratory.

1.7.3 Lab coats and aprons

Lab coats or scrubs are required to be worn by histology personnel. Clean lab coats are to be stored in the coat closet provided. Remove lab coats and leave them on the coat rack on the back of the door to be laundered by linen services. Contaminated lab coats should not be worn out of the laboratory. Remove laboratory coats immediately upon significant contamination.

Non-permeable aprons are required when working with formaldehyde.

1.7.4 Respirators

Representatives from safety administration have determined that when chemical exposures are below permissible levels, a fit-checked respirator is not required. Two types of NIOSH approved nuisance odor masks are provided. Dust/mist masks are to be used when working with chemicals and a charcoal filter mask when working with organic solvents and formalin.

1.7.5 Personal apparel

Open toed shoes are not allowed in the laboratory area. Steel toed shoes are not necessary in this work area.
Confine long hair and loose clothing.

Avoid wearing contact lenses.

1.7.6 Personal housekeeping

Keep the work area clean and uncluttered. Chemicals and equipment should be properly labeled and stored. Clean up the work area at the completion of each operation and at the end of each day. Replace microtome blades in the original box.

1.7.7 Activities which require protection

Activities requiring personal protective equipment delineated for this laboratory include

- working with formaldehyde
- hazardous waste and associated contaminants
- working with acids, bases, or organic material
- changing processors
- grossing and cassetting specimens
- making reagents and solutions

1.8 Use of hoods

Use the hood when performing operations which might result in the release of toxic chemical vapors or dust, and when working with any appreciably volatile substance. Do microwave staining and coverslipping and open heated solutions inside the hood. Gross formalin fixed specimens in the hood, or on a ventilated table. Confirm and document adequate hood performance before use via a reading gauge. Keep materials stored in hoods to a minimum and do not allow them to block vents or air flow.

When working in the hood, move toward the center, avoiding use of the first 6 inches behind the opening. Suggested air velocity is 100 lfm (linear feet per minute).

1.9 Waste disposal

Do not discharge concentrated acids or bases, flammables, highly toxic substances, or heavy metals such as mercury (B-5 fixative) into the sewer. These substances must be recycled or reduced when possible, or placed in appropriate containers to be picked up by safety services. A Hazardous Waste Manifest must accompany waste material.

Formulations of 10% or less of formaldehyde may be discharged to the sewer, unless they exhibit other characteristics of hazardous waste or they are in a high enough volume to generate a problem.

1.10 Working alone

Avoid working alone in the laboratory if the procedures being conducted are hazardous or if you are not properly trained.

2 **TRAINING**

All employees should be aware of the hazards in the work area and measures that should be taken to protect themselves and others in the workplace.

All employees are trained initially when they are hired. There are annual training sessions involving all histology technicians. Training forms are signed by the supervisor and retained in the employees file.

The following information will be covered in training sessions:

2.1 Chemical Hygiene Plan and Safety Manual

- location
- contents
- employee signature

2.2 Material Safety Data Sheets

- location
- instructions for use

2.3 Hazardous chemicals

- handling
- exposure limits and target organs
- labeling
- disposal
- storage
- accidents and spills

2.4 Proper protective equipment

- gloves
- masks

- lab coats, scrubs, and aprons
- safety glasses, goggles
- eye wash stations
- fire extinguishers
- safety showers

HAZARD COMMUNICATION TRAINING CERTIFICATION
AND TRAINING RECORD

I have received hazard communication training as described in the University of Utah Hazard Communication Program.

Date of initial training._____

Employee signature._____

Employee social security number._____

Employee work area._____

I certify that the above named employee has been provided with hazard communication training in accordance with the University of Utah Hazard Communication Program.

Instructor's signature._____

UPDATES AND RETRAINING

Date	Topic	Instructor's initials

3 LABELS AND WARNING SIGNS

Chemical containers must be checked for warning labels. These labels should be maintained in good condition. Any time a chemical is transferred into a different container, the new container must also be appropriately labeled. The label is to include the following:

- The name of the chemical as found on the MSDS.
- Any hazards associated with the chemical or mixture.
- The name and address of the chemical manufacturer or importer.

All solutions must be labeled with the chemical ingredients, preparation date, initials identifying the technician who prepared solution, and expiration date. Known chemical hazards must be on the label.

3.1 Definitions

3.1.1 Label

Any written, printed, or graphic material displayed on or affixed to containers of hazardous chemicals

3.1.2 NFPA Label

National Fire Protection Association code for showing the hazards of materials—information is obtained from the MSDS

- Red fire hazard
- Blue health hazard
- Yellow reactivity
- White specific hazard

3.1.3 Caustic or corrosive

Acids and alkalis may cause burns of skin, eyes and may also cause damage to equipment and storage areas.

3.1.4 Poisons

Poisons may cause acute or chronic effects if inhaled, ingested, or placed in contact with skin.

3.1.5 Carcinogens

Chemicals designated by the Occupational Safety and Health Administration as cancer causing

3.1.6 Flammables

Materials that easily ignite, burn, or serve as fuel for a fire

3.1.7 Explosives

Materials which may explode under special circumstances

3.2 Chemicals and reagents

If the manufacturer's label does not include safety information, apply a NFPA label.

- Codes are listed in paragraph 3.1.2 and on charts posted in the histology laboratory and the flammables room.
- Safety information for each chemical is listed on the MSDS.

When a chemical is received, the label must contain the following information:

- date received
- technician's initials

When chemicals are opened, the label should be dated and initialed.

3.3 Solutions prepared in the laboratory

All solutions prepared in the laboratory must be labeled with the following information:

- name of the solution
- list of chemicals
- percentages (or amounts) of chemicals
- hazard warnings
- date prepared
- preparer's initials
- expiration date

Coplin jar labels should contain:

- name of the solution
- technician's initials
- date the solution was poured into the coplin jar

3.4 Other labels

- Solutions containing formaldehyde must include the yellow “Formaldehyde“ warning label.
- Solutions containing a known carcinogen must be labeled as “Carcinogen”.
- Flammable solutions must be labeled as “Flammable”.
- Solutions containing strong acids or alkalis must be labeled “Corrosive”.

3.5 References

University of Utah Safety Services. “University of Utah hazard communication standard.”

Occupational Safety and Health Administration Federal Regulation #29

4 **Material Safety Data Sheets (MSDSs)**

4.1 Purpose

The Federal Hazard Communication Standard requires that manufacturers and suppliers provide a material safety data sheet for each hazardous substance they produce. There is no standard format for an MSDS; they may vary in length, format, and appearance.

Material Safety Data Sheets are stored alphabetically in a three-ring binder. The chemical MSDSs are separated from the stain MSDSs by index tabs. At the beginning of each index is an alphebetical chemical inventory. Whether a chemical has an MSDS is indicated on the inventory. The binder is to be accessible to all employees in the work area at all times. The inventory and MSDSs are updated as new chemicals and updated sheets arrive.

4.2 Elements of the MSDS

4.2.1 Chemical name

The nomenclature system developed by the International Union of Pure and Applied Chemistry (IUPAC) and Chemical Abstract Service (CAS), or a name which will clearly

identify the chemical for the purpose of conducting a hazard evaluation

4.2.2 Common name

A code name, code number, trade name, brand name or generic name other than its chemical name

4.2.3 Chemical identity

The chemical identity is any chemical or common name which is indicated on the MSDS for the chemical. The name used shall permit cross-references to be made among the required list of hazardous chemicals, the label, and the MSDS.

4.2.4 Manufacturer

The producer of the chemical

4.2.5 Importer (for chemicals produced outside of the United States)

The company within the United States that received and distributed the chemical

4.2.6 Chemical characteristics

The element, chemical compound or mixture of elements and/or compounds

4.2.7 Physical characteristics

Combustible liquid, compressed gas, explosive, flammable, organic peroxide, oxidizer, pyrophoric, unstable (reactive) or water-reactive

4.2.8 Known health effects

- Evidence that acute or chronic health effects may occur in exposed employees
- Health hazards—carcinogen, toxic or highly toxic, reproductive toxin, irritant, corrosive and sensitizer

4.2.9 Primary routes of entry

The means by which material may gain access to the body, for example, inhalation, ingestion or skin contact

4.2.10 Handling precautions
Protective clothing or equipment required when using the substance

4.2.11 Emergency first aid
Procedures in case of contact, ingestion, or other bodily contact

4.2.12 Emergency contact person
The telephone number of the manufacturer

4.2.13 Date of preparation or revision
Date of the last revision of the MSDS

4.3 References

University of Utah Safety Services. “University of Utah hazard communication standard.”

Occupational Safety and Health Administration Federal Regulation #29

5 **Monitoring air samples**

5.1 Policy

In order to assure that histology personnel are not exposed to hazardous fumes above OSHA's permissible exposure limits (PELs), monitoring should be performed if a highly toxic substance is regularly and continuously used. The monitoring is to be performed by a licensed chemical hygienist.

Two test samples must be performed:

- 8 hour time weighted average in parts per million (TWA in ppm)
- 15 minute TWA in ppm

The technicians performing duties with the most exposure to the chemicals will be monitored.

If the sample results are below the exposure limits, monitoring does not need to be continued. If procedures change

in the future and exposure increases, then technicians should be monitored again.

If the sample results come back above the PELs:

- Procedure changes must be made to lower exposure.
- Personal protective equipment, such as respirators, must be made available and used. Respirators must be provided by the chemical hygienist only. The employee must be test fitted and trained in using the respirator.
- Medical monitoring and physicals will be provided at no cost to the employee.
- Monitoring will be provided at 6 month intervals.

5.2 Procedure

1. Contact safety services.
2. Give the chemical name to be monitored and the affected employees' names.
3. The chemical hygienist will make the appointment. Be sure the duties or procedures performed include those with the highest exposure to the hazardous agent.
4. On the day of the monitoring the hygienist will arrive and place the monitoring device on the technician's lab coat lapel. The device is not worn during coffee or lunch breaks.
5. At the conclusion of the monitoring the hygienist will pack up the monitoring devices which will be sent to an outside laboratory for the results.
6. When the results are received, copies will be sent to the laboratory supervisor, lab manager, and the employee. The original is retained by safety services.
7. Employees have the right to request monitoring if over exposure is suspected. The request should be made to the histology supervisor.

5.3 Reference

Occupational Safety and Health Association regulations.

6 **Spills**

6.1 General procedure

1. Attend to any persons who may have been contaminated.

2. Notify persons in the immediate area and evacuate all nonessential personnel from the spill area.
3. Review the substance's MSDS.
4. If the spilled material is flammable, turn off ignition and heat sources.
5. Avoid breathing the vapors of spilled material.
6. Obtain cleanup supplies.
7. During cleanup wear appropriate apparel: lab coat or apron, gloves, goggles, and mask. Masks are not a substitute for respirators, and are only recommended for use when the airborne contaminate is under the PEL.
8. Dispose of gloves, aprons, and masks in a bag.
9. Notify Safety Services of the spill.

6.2 Small spills other than acids

1. Use spill pads to absorb the liquid.
2. If the spill is a flammable solvent, place pad in front of a fume vent or in a hood until dry. If the spill is a stain or non-hazardous solution, place the pad in a plastic bag and discard.

6.3 Large spills

If the spill is too large or too dangerous to clean up without assistance, do the following:

1. Contain the spill if possible.
2. Call Safety Services and report this information:
   - your name
   - telephone number
   - exact location of the spill
   - nature of the spill
   - any illness or injury due to spill

6.4 Acids: absorbent spill kit

1. Pour the absorbent material around the edge of the spill to stop it from spreading.
2. Pour generous amounts of the absorbent material on the spill until the top layer remains dry.
3. Sprinkle the appropriate neutralizing powder on the absorbent material.

4. With the dustpan provided, mix the absorbent and the neutralizer.
5. With the dustpan and the brush, sweep up the mixture and place it in the clear plastic bag.
6. Label bag as hazardous waste and clean off the brush and the dustpan.
7. Call safety services for pickup.

6.5 Kolor-safe™ liquid neutralizer

1. Use proper protective clothing and equipment.
2. Soak up the spill by placing absorbent pads on the spill.
3. Spray or slowly pour the correct neutralizer on the spill.
   - For bases, use the red solution. The color indicator will change from red to blue while neutralizing. Continue to apply neutralizer until the color turns a yellow color. Do not over neutralize.
   - For acids, use the purple solution. The color indicator will change from purple to yellow while neutralizing. Continue to apply neutralizer until the color turns a reddish color. Do not over neutralize.
4. Carefully pick up the pads and place them in a plastic bag, labeled "hazardous waste".
5. Call safety services for disposal.

6.6 Flammables

1. Often, the vapors are what burn, not the liquid.
2. Contain the spill by laying absorbent spill pads around and on the spill area.
3. If the spill is small, place the pads in a pan and place the pan in the fume hood.
4. Notify safety services.

6.7 Solids

1. Sweep up the spill, but try not to cause dust to fly into the air. Place the material in a bag.
2. List the substance on the waste manifest for pickup by safety services.

6.8 Mercury

1. Wear gloves to avoid skin contact with elemental mercury.

2. Ventilate the area well to remove mercury vapors.
3. Clean up the spill with a pipette or sweeper (two pieces of paper will work).
4. Place elemental mercury waste in a labeled plastic container, along with the devices used to clean it up. Dispose of the gloves.
5. Call safety services for disposal.

6.9 Formaldehyde: SPILL-X-FP™

Use a 1.85lb container to 2 gallons (less 8 oz) of 10% formalin solution.

1. Use appropriate protective equipment.
2. Contain the spill by pouring SPILL-X-FP around the edges of spill, then apply inward. Be careful not to splash. Cover spill evenly with the agent.
3. Carefully mix the SPILL-X-FP with the formalin spill for best polymerization.
4. Wait 15 minutes. The solution may solidify.
5. Sweep the spill into a bag. Wash off the broom and dustpan.
6. Label the bag as hazardous waste and notify safety services.

6.10 Blood and body fluids: VITAL1™

1. Wear gloves to avoid skin contact.
2. Completely cover the spill with the contents of white pouch (chlorinated absorbent beads).
3. When the spill begins to solidify, use the pick-up shovel to place the substance into the white plastic bag. Seal the bag with ties.
4. Wipe up area with the germicidal towel (sani-cloth) provided.
5. Place all materials (white bag, shovel, germicidal towlette, wiping cloth, and gloves) into the red bag and tie it closed.
6. Dispose of the bag in a biohazard waste container.
7. Wipe hands with an antiseptic hand wipe towlette and allow to air dry.
8. File an incident report.

6.11 References

National Research Council. 1988. *Prudent practices for handling hazardous chemicals in laboratories*. Washington D.C.: National Academy Press. 233-235

Spill kit instructions: Kolor-Safe, Fisher Absorbent, SPILL-X-FS, Vital 1.

University of Utah Safety Services. *University of Utah safety & health manual*.

7 **Storage and handling**

7.1 General procedure

Good laboratory practice should be observed even when handling substances of no known significant hazard. Minimize exposure by working in an exhaust hood, wearing eye and hand protection, and a laboratory coat or an apron.

7.2 Special handling

For substances that require special handling, observe the following procedures:

1. Protect the hands and forearms by wearing either gloves and a lab coat or gloves and sleeves.
2. Procedures involving volatile toxic substances, vapors and fumes should be performed in the hood. The hood should have a face velocity of at least 60 linear feet per minute. Avoid breathing dust or fumes.
3. Wash hands immediately after use.
4. Clean up the area immediately after the procedure to avoid exposing coworkers.

7.3 Corrosives

Corrosives are strong acids or bases. They may be liquid or solid. Inhalation of the vapors or mists of these substances can cause severe bronchial irritation. These chemicals erode the skin and respiratory epithelium and are particularly harmful to the eyes. Corrosives are also damaging to steel.

1. Wear protective clothing when working with corrosives.
2. Acids should be stored separate from other chemicals. The place where acids are stored should be designated as “acid

cabinet". They should be kept on low shelves to minimize the danger of containers falling.

3. Acid carriers are provided and must be used for any bottles over 1 liter in size.
4. When mixing solutions, always add the acid slowly to the water. Never pour water into concentrated acids or bases.
5. Do not mix incompatible chemicals. If in doubt check the MSDS, or the Merk index.

7.4 Flammables

A flammable is any liquid having a flashpoint below 100°F. When working with flammables, the following guidelines must be observed:

1. Quantities of one gallon or more must be stored in a safety can with a spring closing lid and spout cover.
2. Not more than a total of 10 gallons of flammable liquid may be stored outside of an approved flammable liquid storage cabinet or room.
3. Do not store flammables in areas exposed to direct sunlight.
4. Do not store combustibles (e.g., cardboard boxes) with flammables.
5. Do not store flammable substances in refrigerators unless the refrigerator is explosion proof.
6. Tissue processors and similar automatic equipment using flammable or combustible reagents should be operated at least 5 feet away from heat sources such as embedding centers.
7. When dispensing alcohol from a 55 gallon drum, the drum should be upright and grounded. Alcohol is dispensed into 5 gallon safety cans.
8. Label all flammables with a "Flammables" label.

7.5 Highly reactive chemicals and explosives

When the term "routine chemical reaction" is used, it usually refers to a relatively slow or easily controllable reaction. When working with the following highly reactive chemicals, special procedures must be taken:.

1. Picric acid crystals must
   - always have 10% water added to the container—it is explosive when dry,.
   - be kept out of contact with metal,
   - never be used near an open flame.
2. Benzoyl peroxide is
   - easily ignited and sensitive to shock,
   - decomposes spontaneously at temperatures above 50°C,
   - can be desensitized by the addition of 20% water.
3. When using anhydrous aluminum chloride
   - completely enclose the bottle in a heavy towel and open slowly,
   - add water slowly.
4. Ammonia
   - becomes explosive when mixed with iodine,
   - reacts with hypochlorite to become chlorine,
   - may become explosive with silver nitrate and should be disposed of after use.
5. Chromic acid (chromium trioxide) will react with alcohol and other flammables.
6. Dry ice and liquid nitrogen do not keep in sealed containers; the carbon dioxide build-up will cause the container to burst. Containers specifically designed for such storage should be used.

7.6 Carcinogens

The Occupational Safety and Health Administration has established procedures to be followed when working with known carcinogens. Anyone working with the materials on the list that follows should be familiar with the necessary approvals, training, working conditions, monitoring, record-keeping, and medical surveillance.

7.6.1 Known carcinogens

- aniline
- chloroform
- chromium compounds
  - chromic acid
  - potassium dichromate
- formaldehyde

Carcinogenic stains

- auramine o, CI 41000
- basic fuchsin (pararosaniline hydrochloride), CI 42500
- chlorazol black e, CI 30235
- ponceau de xylidine (ponceau 2R), CI 16150

Substances that cause cancer in animals (no human data)

- acridine orange
- basic fuchsin (rosaniline hydrochloride, CI 42510
- Congo red
- crystal violet (gentian violet),
- yosin y, CI 45380
- fast green FSF, CI 42053
- hematoxylin, CI 75290
- light green SF yellowish, CI 42095
- orange g, CI 16230
- rhodamine B, CI 45170
- Sudan III, CI 26100
- Sudan IV, CI 26105

7.6.2 Safety procedures

When working with carcinogens observe the following guidelines:

- Wear protective clothing including gloves, goggles, and a lab coat. Wear a rubber or plastic apron when working with liquids.
- Work only in a well ventilated area, preferably in a fume hood. The hood sash should be pulled to the mark which indicates 150 fpm face velocity.
- Clean up carefully and wash hands after procedures.
- Dispose of contaminated paper towels, weigh boats, and other materials in a sealed container labeled “Cancer Suspect Agent”.
- Read the MSDS for the substance being used.

7.7 References

University of Utah Safety Services. *University of Utah safety & health manual*.

National Research Council. 1988. *Prudent practices for handling hazardous chemicals in laboratories*. Washington D.C.: National Academy Press. 233-235

Montgomery, Rudy. 1989. *Right to know*. NSH Workshop.

8 **Waste disposal and reduction**

8.1 Containers

Only appropriate containers should be used as receptacles of hazardous waste. All containers must have secure lids that will not leak when inverted.

Fifty-five gallon metal drums should be used to contain

- xylene
- acetone
- alcohol
- chloroform

Glass containers should be used for

- b-5 fixative (liquid state, not reduced)
- any solution containing chemicals corrosive to metal
- glycol methacrylate waste, polymerized to a solid state and disposed of in the trash

8.2 Labels

"Hazardous Waste" tags must be attached to each container of waste. Copies are retained by safety services and the histology office. The following information must be included on the tags:

- chemical constituents (percentage of the total should be listed
- name of the person requesting disposal
- room number and building
- Environmental Protection Agency hazardous waste number
- date of disposal
- description of waste
- quantity
- hazard class
- signatures

8.3 Disposal procedures

EPA regulations require that “containers holding hazardous waste must always be closed during storage, except when it is necessary to add or remove waste” (42cfr265.1739(a)). *Closed* means having a lid which prevents waste from entering the atmosphere.

Never dispose of strong oxidizers and acids together. If you have any questions call safety services for help.

1. Place the tag on the container as soon as any waste has been added to it.
2. On the tag, fill out the name of person responsible for waste identification, phone number, department, building, and room number.
3. When the container is full, fill out the rest of the tag.
4. Remove the white copy and mail it to environmental health and safety.
5. Pack glass bottles in cardboard boxes.

8.4 References

“University of Utah Hazardous Material Disposal Procedures.” January 1993

Academy Press. 1981. *Prudent practices for disposal of chemicals from laboratories*.