

## **SPECIAL SAFETY REGULATIONS BY LABORATORY SECTION**

### **INTRODUCTION:**

Many laboratory sections require special safety regulations due to the nature of the equipment, specimens or reagents used. The following regulations are limited in scope to the particular laboratory area.

### **GOALS:**

The procedures and regulations that follow are intended to ensure that hazards are minimized in the Pathology Department.

### **ENFORCEMENT:**

Supervisors and Section Directors are responsible for enforcement of the regulations.

### **HEMATOLOGY LABORATORY- FLOW CYTOMETRY- LASER SAFETY:**

Lasers used in modern, clinical flow cytometers are low power, totally enclosed within the instrument and are physically and electronically interlocked to prevent operator exposure to damaging laser radiation. The following general laser precautions and information can educate workers to the nature of the device, should service personnel override the interlocks for necessary maintenance.

The laser is a unique light source and it exhibits characteristics which are different from conventional light sources. Its safe use depends on the user becoming aware of these characteristics and treating the instrument accordingly. The high-energy output of the beam passing directly into the eye can cause serious damage with the possible loss of vision. Because it remains coherent, the beam might also cause damage to the eyes if it is contacted indirectly from reflective surfaces. Many light-sensitive elements can be damaged by direct exposure to the beam; e.g., the light-sensing element in video camera, photo-multipliers or photo-diodes. With some lasers, the energy of the beam is intense enough to ignite volatile substances from some distance.

### **Regulations:**

- a. In accordance with current manufacturer guidelines, operators may never bypass interlock devices during the normal operation and calibration of the instrument.
- b. If service, which requires bypass of the interlocks is required, hand jewelry and shiny watches must be removed before working in the optical bench area or in the laser path. Shiny, reflective tools must never be used on the optical bench area without first shutting the laser down.

- c. In maintenance, one must never look directly at the laser beam.
- d. Laser warning signs (although not required) are to be posted on the lab door to sensitize personnel as to the potential for hazard.

**Radionucleide Laboratory:**

No longer uses isotopes.

**TB Mycology:**

- a. All work involving infectious material in which an aerosol may be created (e.g., pouring of specimens, pipetting, vortexing) must be performed in a biological safety cabinet (BSC).  
Centrifugation on infectious material must be done using a double closure system (a screw-capped or rubber-stoppered centrifuge container enclosed within a sealed centrifuge bucket). The containers are to be opened only in the BSC.
- b. The blower on the cabinet should be turned on and allowed to warm up for 15 minutes before using.
- c. Within the BSC, equipment and supplies must be arranged in such a manner that hands and arms do not pass over contaminated surfaces (such as inoculated slides).
- d. At the end of the day, the BSC blower is turned off. After waiting five minutes for the air to settle, the door to the cabinet is lifted so that the entire cabinet is wiped down with phenolic disinfectant.
- e. The UV light is left on in the cabinet overnight to provide additional germicidal protection.
- f. The cabinet is professionally inspected and certified annually.
- g. Contaminated disposable non-glass and non-sharp items (used in the processing of specimens or in the inoculation of media or test substrates (e.g., swabs gauze pads) and supernatant fluid for specimens) are discarded into an autoclave pan or other container containing Amphyl disinfectant. The pan is covered, taped securely, and autoclaved at the end of the day. The contents are then disposed with the other biohazard wastes.
- h. Disposable sharps (e.g., syringes, glass pipettes, slides) are discarded into the sharps containers.

l. Other contaminated disposable items, such as specimen containers and gloves, are discarded in the standard biohazard waste stream.

j. Reusable contaminated glassware with closed top (i.e., screw-top test tubes) are placed in a separate bucket that is lined with a biohazard bag. The bag is autoclaved and the contents are well-washed and sterilized before being placed back into service.

k. Tissue grinders are placed in a autoclave pan containing Amphyl disinfectant. After autoclaving, the grinders are properly washed and sterilized before placing back into service. (Alternatively, disposable tissue grinders may be used and discarded appropriately.)

l. Micro-incinerators are used instead of Bunsen burners.

m. Spills within the BSC: Spills of infectious material are to be immediately flooded with Amphyl disinfectant, covered with paper towels, and allowed to soak for at least 15 minutes before wiping up. Normal protective equipment—gloves and barrier coat—are to be worn when handling these spills.

If the spill involves broken glass, one must pick the glass with forceps or other means— not with unprotected hands.

Spills outside of the BSC: Vacate the room immediately and close off for at least 30 minutes ( to allow any aerosolized particles to settle). Then, using an N95 respirator and normal protective gown and gloves, enter the room to perform the cleanup (proceed as above).

n. Heat -fixing of slides is only to be done in the BSC or on an electric slide warmer , equipped with a cover.

o. After inoculation of the BACTEC vial, and each time material is withdrawn from the vials, the tops must be disinfected with Amphyl.

p. The fan and the UV light on the BACTEC hood must always be on when testing vials.

q. Following completion of a run on the BACTEC instrument, the hood must remain closed for 5 minutes before removing any vials.

r. Culture plates with filamentous growth, compatible with a dimorphic fungus, must be taped shut and opened only in the BSC.

s. Slide cultures must never be set up on respiratory dimorphic fungi.

## **VIROLOGY:**

- a. Inoculation or processing of specimens should be carried out in Biological Safety Hoods. Hood should be turned on 10 minutes before use.
- b. Contaminated scissors and forceps are placed into covered metal canister for autoclaving before washing and sterilization for reuse.
- c. Contaminated rubber-tipped policemen are placed into a beaker of bleach, then placed into a covered plastic pan with 1:10 dilute bleach for autoclaving before washing and sterilization for reuse.
- d. Contaminated mortars and pestles are placed in a covered plastic pan with 1:10 dilute bleach for autoclaving before washing and sterilization for reuse.
- e. All throw-away, non-sharp, contaminated materials are placed into a covered plastic pan with 1:10 dilute bleach before discarding.
- f. All throw-away sharp materials are placed in to an approved sharps container; when no more than 3/4 full it is capped to be disposed by the vendor.
- g. Sodium bicarbonate must be filtered using a syringe to draw up the reagent, then the needle is removed using the slot on a sharps container so that a filter can be attached to the syringe. This procedure is NEVER performed on clinical specimens.
- h. All procedures involving clinical specimens not performed under the hood, such as separating serum and serological tests, are performed behind a plastic shield.
- i. All specimens containing infectious or possibly infectious agents are centrifuged in glass or plastic tubes with tight-fitting caps.
- j. All infectious or possibly infectious materials discarded outside of the biohazard hoods are placed into orange bags for pick-up by pathology personnel and autoclaved before proper disposal.
- k. Cover all receptacles which contain formalin. Dispose of all formalin by pouring down the drain, followed by a rinse of water. Both measures are to minimize the presence of formaldehyde fumes.

## **MORGUE:**

Autopsy precautions for all autopsies:

### **Highly Infectious Cases:**

To limit traffic and potential exposure, highly infectious cases, including Mycobacterium tuberculosis (TB), AIDS, Hepatitis and C.J. are to be performed in the small autopsy room. Procedures and precautions are the same, except for the procedures for fixing C.J. tissue and CJ disease cleanup (see section specific to CJ disease which follow these rules). Prosectors should contact staff when they are requested to autopsy these patients. There is routine staff attendance and supervision at these procedures.

- a. The Pathologist is to review the chart, ascertain the adequate permission, and mentally ascertain the approach that he will use in performance of the autopsy, although all autopsies are approached with universal precautions.
  
- b. Morgue personnel are to be instructed in the special precautions needed due to the state requirements for the appropriate labeling of possibly infectious bodies.

c. Apparel, including hair covers , masks, goggles, splash suits (including head covers, shirts, pants, and shoe coverings) are to be worn during the autopsy and disposed of in plastic bags after completion of the autopsy. The prosector should double glove before the autopsy and at the completion of the autopsy remove the outer gloves first, inspect the inner gloves for tears, cut, or external staining and then remove the inner gloves and discard. No tissues, jars, or specimen containers are to be handled after gloves have been removed. If cuts are found in the gloves, inspect the hands for cuts or lesions, if found, report the exposure via the employee accident procedure and report immediately to employee health (or emergency room).

d. One autopsy assistant will assist the prosector in the autopsy room, but no other personnel will be admitted without the permission of the prosector. All individuals with permission to be in the autopsy room will be properly attired. Because of this requirement of proper attire and the expense of the suits, the number of individuals should be at a minimum.

e. The detail of dissection is at the discretion of the prosector, but dissection at the autopsy table should be minimal with removal of organs, and careful slicing so that fixation will be adequate. All tissues removed are to be fixed in 10% formalin for 5-7 days before further dissection, and the morgue personnel are to change the formalin at day 3 for optimal fixation. Prosectors are encouraged to take small pieces of tissue for histology at the autopsy table for best fixation rather than taking pieces from the large pieces later. It is not required that the entire liver, spleen, or grossly normal organs be fixed, but representative slices should be fixed for subsequent review staff. Any tissue not saved is to be placed in double plastic bags and placed in the body cavity prior to sewing the body.

f. It is recommended that the Stryker saw be used only to open cranium , and the ribs should be cut with the rib cutter thereby decreasing the bone dust aerosols.

g. The cranium is to be opened by the autopsy assistant and the saw and cranium are to be placed in a clear plastic bag before any cutting is done. By placing the head and saw in the bag ( a hole is placed in the bag for the saw cord), aerosols of bone dust are kept to a minimum. The brain, after removal, is fixed in 10% formalin prior to neuropathologic examination.

h. At the completion of the gross dissection, all tissues not fixed are double bagged and placed in the body cavity and the body cavity and cranium are closed with sutures to minimize fluid loss. The body is rinsed with copious amounts of water, which is allowed to drain into the sewerage pipes.

**For known highly infectious cases: The body is then placed in the zippered polyethylene body bag (exception: stillborn who are wrapped in a small shroud), placed on a roller, and labeled with an appropriate label--CAUTION-TB, AIDS, HEPATITIS, etc. The body is then placed in the walk-in box nearest the morgue office and a sign placed on the door with an appropriate label--TB, AIDS, HEPATITIS, etc.**

- i. The prosector may leave before the final body preparation is completed, but he is to remove the protective clothing before leaving the morgue premises, and all protective clothing is to be placed in the plastic bags for proper disposal.
- j. All instruments are to be washed with soap and water and then soaked in a 1/10 dilute bleach solution for at least four hours. The table and outside of the formalin storage containers are to be washed with soap and water and then flooded with a 1/10 dilute bleach solution. Bleach is added to the mop bucket, and the floor mopped with this solution. After cleaning the room, the autopsy assistant is to remove his protective clothing, place it in a plastic bag, and dispose of properly with the other clothing worn by the prosector.
- k. Material removed from the patient (i.e. catheters, IV. tubing, etc.) are to be placed in the plastic bags for disposal -- Sharps must be placed in the sharps container.

l. For known highly infectious cases, after the instruments, containers and room is cleaned , a sign is placed on the door restricting admittance for a 24 hr period (except for the autopsy assistant who is to remove the instruments from the bleach solution after 4 hrs.).

m. The plastic bags are labeled as contaminated and are placed in the refrigerator for subsequent disposal (i.e. cremation).

n. After fixation of the tissues for 5-7 days, they may be further examined and dissected in the small autopsy room. When the prosector is through with the tissues and there is no need to save the tissue further, it is to be placed in double plastic bags, labeled, and prepared for disposal according to MCLA policy.

o. Barrier clothing for performance of these autopsies is available from the autopsy assistants on request. The materials are stored under lock to prevent theft.

p. When funeral homes request the remains on highly-infectious cases, morgue personnel are to inform the funeral director of the disease state of the deceased, so that appropriate body wraps may be brought for transport.

#### **HISTOPATHOLOGY:**

##### **Safety Regulations for the Sectioning of Unfixed Tissue for Frozen Section:**

1. Handle all tissues with gloves. Work on a surgical drape which is to be discarded later in the orange bio-hazard bag. (Or returned to surgery if they desire.)
2. In very unusual cases, with staff approval, (such as an AIDS patient with cryptococcus infection), the tissue block may be heated in a beaker of formalin until steaming or near boiling. If this technique is performed, it should be done in a fume hood or with adequate ventilation. This pasteurizes the tissue.
3. Section the tissue as usual in the cryostat. Aerosols used for fast freezing are contraindicated and are not used.
4. Mount sections on slides prepared with poly- L-lysine. Press the slide firmly against the section on the frozen-section-knife-surface.
5. Clean up and sterilize instruments and contaminated surfaces as follows:

a. Cryostat - Daily wipe all surfaces with a towel or sponge which has been soaked in absolute ethanol.

b. Cryostat Knife Holder - monthly soak in pan of ethanol for 15 min.

c. Dissection instruments - Daily soak in 1/10 dilute bleach for 15 min. then wash well in tap water.

d. Bench surfaces- Daily use standard bench disinfection technique (see page 2.7).

**Additional section-specific instructions for handling cases of suspected Creutzfeld-Jakob disease (from patients with a history of subacute or chronic dementia).**

Formalin fixation alone is ineffective in reducing the infectivity of the transmissible agent of Creutzfeld-Jakob disease (CJD). Methods of tissue decontamination previously recommended such as autoclaving, sodium hydroxide or bleach may not be effective or may produce extreme cytologic artifact and are not acceptable for histologic purposes.

Specimens immersed in formalin alone, phenol/ formalin or phenol / formic acid have higher infectivity levels than those with formic acid / formalin pretreatment. The addition of a one hour formic acid immersion step in the handling of tissues from any patient with subacute or chronic dementia of unknown etiology appears to be the most effective method thus far for both effective reduction in infectivity and preservation of histological detail.

Gloves and protective eye shields are to be worn at all stages of handling suspected tissues, including sectioning and staining procedures. All suspect tissues should be considered infectious throughout all procedures— including fixation, embedding, sectioning staining and mounting.

1. At autopsy: Clean and sterilize the autopsy table and instruments using 1/10 dilute bleach— soaking for at least one hour.
2. Then autoclave instruments for at least 60 minutes at 132° C (min).

2. In the Histopathology lab:

Prior to tissue processing

- a. Place autopsy tissues and brain biopsies from patients with subacute chronic dementia of unknown etiology in 10% neutral buffered formalin for 48 hours.
- b. Place the fixed tissue sections (not more than 4-5 mm. in thickness) in 50-100 ml of formic acid (>96% ACS grade) for one hour.
- c. Re-immerses the tissues in 10% buffered neutral formalin for 48 hrs, followed by tissue processing.
- d. If tissues are positive for CJD, the brain is cremated.

Processing:

- a. After sectioning of paraffin blocks, wipe the exterior of the microtome and flotation bath with 1/10 dilute bleach and rinse with water.

- b. Fluids must be steam autoclaved for at least one hour at 132-134° C per the normal biohazard procedure. We routinely autoclave wastes or 90 minutes.
- c. Decontaminate the work area and instruments with 1/10 dilute bleach (not diluted) for at least one hour, wash well with water and dry.
- d. As usual, place trimmed wax shavings, gauze and gloves in the biohazard wastes for steam autoclaving prior to disposal.
- e. Place paraffin blocks and slides in sealed bags (or other containers) labeled with regard to the possibility of CJD. Label slides as infectious.

### **Regulations for Formaldehyde :**

Formaldehyde is classified as a category 2a carcinogen, associated with upper -respiratory tract neoplasms. The widespread use of low concentration formaldehyde as a tissue fixative and disinfectant, precludes regulation that would normally be used for a category 2a carcinogen. Special OSHA regulations allow the use of formaldehyde, so long as work practices and engineering controls are sufficient to maintain employee exposure levels below threshold limits (see the Chemical Management Plan).

The follow regulations and practices ensure that this condition will be met in the Anatomic Pathology section of the department:

1. Formaldehyde is to be purchased in low concentration (pre-diluted form). This precludes exposure during mixing of highly concentrated material. Small quantities of 37% formaldehyde are maintained for special uses.
2. Formalin is to be purchased in convenient , easy-pour, disposable containers.
3. Materials fixed in formalin are examined and sectioned under the Grosslab□ fume hood. All extraneous formaldehyde is decanted in this device as well (with continuous water flow to flush the device).
4. Empty containers are dipped in a 1% solution of Potassium Permanganate prior to disposal.
5. The formalin extraction device is run every night from 1600h to 0700h.
6. Lidded garbage cans are used for disposal of specimen containers (after dipping in permanganate).
7. Sorting of tissue cassettes is done following rinsing with copious amounts of tap water to remove the surface formaldehyde.

8. Special formaldehyde neutralizing spill control substances (e.g. Spill XFP) are purchased, available and used for spill control.
9. The unit personnel are monitored at least once per calendar year for formaldehyde exposure at a 15 min. (STEL) and an 8hr (PEL) level. The STEL levels are to be measured at the likely peak of the daily exposure (e.g. "cutting down").
10. Medical Surveillance activities for possible formalin-related health problems are coordinated via the Employee Health Clinic. Employees receive annual training in the signs and symptoms of over-exposure to formaldehyde. Surveillance is also available if monitors exceed the action limits or following an acute accidental exposure.

**BLOOD BANK:**

**Policy regarding the storage and use of blood collected for autologous transfusion that tests positive for infectious disease**

The Blood Center for Southeast La. will not release, to the MCL Blood Bank, any unit of blood for autologous transfusion if it tests positive for any infectious disease (except for RPR and Hepatitis B core antibody positive units).

This precludes previous policies regarding storage and disposal of infectious units collected by the Blood Bank. Effective 12/90.