

# **Biohazard protocol- Williams Laboratory Higgins Hall 457 and 458**

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## **Biosafety Level 2**

### **Work Practices**

Adopted from *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition*. HHS Publication (CDC), 2007.

#### **STANDARD MICROBIOLOGICAL PROCEDURES - BSL 2**

1. Access to the lab is restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after handling infectious agents, after removing gloves, and before leaving the lab.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics is not permitted in work areas. Persons who wear contact lenses should also wear goggles or a face shield.
4. No mouth pipetting.
5. All procedures are performed to minimize the creation of splashes and aerosols.
6. Work surfaces are decontaminated at least once a day and after any spill of viable material.
7. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container and closed for transport from the laboratory.

#### **SPECIAL PRACTICES - BSL 2**

1. Laboratory director limits access to labs when work with infectious agents is in progress. The laboratory director determines who may or may not enter labs.
2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific entry requirements enter the laboratory.
3. A hazard warning sign is posted on the access door to the laboratory work area where infectious agents are being handled.
4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g. hepatitis B). See Appendix C Boston College Biohazard Manual for more detailed information.
5. When appropriate, baseline serum samples are collected and stored.
6. A biosafety manual is prepared and adopted.

Laboratory personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. All employees will be tested with a yearly refresher quiz.

## General work practices for BL2+ Laboratories

### 1. Access

- A. Access is limited to authorized, trained personnel. Both the BL2+ sorter (Higgins Hall 458) and BL2 culture (Higgins Hall 457) rooms will be equipped with doors with keys pads. In addition, there will be an additional door placed outside the 2 rooms with limited access. All visitors must be informed of the nature and risk of exposure to the agents in the laboratory.
- B. Environmental Services will be permitted in the BL2+ laboratories before 9a.m. and after 5 p.m. each day for the purpose of cleaning floors and emptying wastebaskets. All involved personnel will receive special training prior to working in these labs. No work with infectious agents will be performed while cleaning is in progress.
- C. Access will be limited by locks on the doors and doors will **remain closed** while work is being performed.
- D. There will be areas designated as "clean" and "dirty". In both room 457 and 458 there will be anti-rooms ("clean") and they will be marked appropriately. Passing into the dirty area will required removing of "clean" white lab coat. Passing back to the clean area will require removal of disposable blue gown and hand washing.

### 2. Protective clothing

- A. Lab coats and gowns:
  1. For entry to and work in BL2+ labs everyone must wear a disposable blue solid-front gown. This gown must be removed prior to exiting to the clean area of lab. These gowns worn exclusively in the BL2+ labs will be autoclaved prior disposal (disposal in orange biohazard bags).
- B. Gloves
  1. When handling infectious agents, double gloving with latex or nitrile gloves is required. If the gloves are possibly contaminated, the outer gloves will be removed prior to removing hands from the biosafety cabinet and discarded in the orange bag inside the biosafety cabinet.
  2. Before hand washing and prior to exiting the laboratory, gloves are disposed in glove disposal container (double lined with orange biohazard bag) next to the hand-wash sink.
  3. If (because of the nature of the material that you are carrying) you must wear gloves outside any lab, only wear **ONE glove** and use your ungloved hand to open doors, etc.
- C. Eye protection

Includes glasses, goggles, or face shields. Eye protection is always required when a procedure is likely to generate splashes or the creation of aerosols. Contact lenses are discouraged, but for those who do wear contact lenses, glasses, or goggles are especially important in BL2+ laboratories.

### 3. Exiting laboratory

- A. Lab blue gowns will be hung in "dirty" area of laboratory.
- B. **Hands will be washed** at exit sink regardless of the time spent in BL2+ laboratory.

**Waste Overview**

	<b>NON-HAZARDOUS SOLID WASTE</b>	<b>INFECTIOUS SOLID WASTE</b>	<b>SHARPS</b>	<b>INFECTIOUS LIQUID WASTE</b>
<b>EXAMPLES OF WASTE</b>	Pipets, plasticware, paper, glass (unbroken), gloves, etc. which have not been in contact with infectious material.	Pipet tips, plasticware, paper, glass (unbroken), gloves, etc. which have, or MAY have, come in contact with blood.	ALL broken glass, Pasteur pipets, needles, razor blades, and anything else sharp that could potentially puncture.	Ficoll/blood tubes. Flow cytometry tubes. Liquid waste from vacuum traps.
<b>HOW TO HANDLE WASTE IN LABORATORIES</b>	Place in fly-tight waterproof container <del>double-lined with red bag.</del> Place <i>regular waste</i> (like packaging material) into waste basket	Place in plastic bucket lined with <b>orange biohazard autoclave bag</b> . Close bag securely before placing on cart in a BL2+ lab.	Place in a standard <b>sharps container</b> . Bring full recyclable container to the designated rack in the receiving area in exchange for an empty container. Small red biohazard sharps containers are closed and placed in orange biohazard autoclave bag.	<b>Chemical disinfection</b> with at least <b>10% bleach</b> .  blood tubes: close tightly and dispose as infectious solid waste.  Flow cytometry tubes: Place tubes in a water tight, 5 gallon sharps container.

<p><b>HOW WASTE IS DISPOSED OF</b></p>	<p>Close securely before putting into <b>double-lined (red bag)</b>. Waste baskets with <i>regular waste</i> will be emptied by Environmental Services staff each evening when labs are cleaned.</p>	<p>Waste will be <b>autoclaved</b> and disposed of as regulated medical waste.</p>	<p>Call the Service Response Center at XXX to let Biosystems know they have to do a pickup at Higgins.</p>	<p>Liquid waste: Sink dispose after bleach (let sit for <b>at least 20min</b> in bleach after infectious material is added). Flow cytometry tubes: When full, double red bag and place in a burn box for disposal without auto-claving.</p>
<p><b>SPECIAL INSTRUCTIONS</b></p>	<p>Serum bottles need to be disposed as medical waste (burn box).</p>	<p>Minimize the amount of liquid you put in these bags.</p>	<p>No bleach or other liquid in sharps containers.</p>	

## Biosafety Procedure 1

### Disposal of solid waste

#### DISPOSAL OF SOLID INFECTIOUS WASTE

##### Examples of waste:

Pipet tips, plasticware, paper, unbroken glass, gloves, etc. which MAY have come in contact with blood, cultured cells, or supernatants of cultured cells of a human or monkey origin, blue gowns.

##### How handled:

- Line a plastic bucket with an **orange autoclave bag**. Dispose of solid waste in this bucket. When full (or at the end of the day) close the bag securely with the rubber bands provided with the bags. Waste must not overflow the top of the bucket. Autoclave the orange bags.
- Place gloves used in the tissue culture labs in a bucket with an orange autoclave bag or in a glove waste container **double-lined** with orange biohazard bags. Glove waste containers are located next to the handwash sinks in the tissue culture labs. When glove waste is full, close bag with rubberband or tape and autoclave. Reline the glove waste container with two orange biohazard bags.
- Blue gowns are disposed of in a big cardboard box **double-lined** with orange biohazard bags. When gown disposal boxes are  $\frac{3}{4}$  full, close the bag with rubberband or tape and autoclave cart.

##### How disposed of:

After autoclaving the waste is placed in a double-lined (red bag) burn box and disposed of as regulated medical waste. See Appendix C Boston College Biohazard Manual for more detailed information.

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#### DISPOSAL OF SOLID NON-INFECTIOUS MEDICAL WASTE

##### Examples of waste:

Pipets, plasticware, paper, gloves, etc. which have not been in contact with infectious material. For unbroken glass see "Special Note" page 9.

##### How handled:

Line a molecular waste container with **two red bags**. Dispose of solid waste in this bucket so that the lid can still closely fit (fly-tight). When not working at the molecular bench or when you do need to use the container, keep the lid closed. When full, close the bag securely with rubber bands or tape. Bring the waste in a secondary containment (waste container) to a burn box. Put the closed filled red bags into a burn box double-lined with red bags. When the burn box is filled, close the red bags and tape the burn box close.

##### SERUM bottles:

Empty serum bottles are disposed of in **burn boxes double-lined with red bags**. The burn boxes in the tissue culture lab are located next to the gown disposal boxes. When the burn box is filled, close the red bags and tape the burn box close.

## Biosafety Procedure 2

### Disposal of sharps

Generally avoid using sharps in the BL2 tissue culture lab.

**Examples of waste:**

All broken or chipped glassware, pasture pipets, needles, razor blades, or anything else that could potentially puncture.

**How handled:**

Place in a *standard red sharps container*. Do not overfill the sharps containers. Replace them when they are  $\frac{3}{4}$  full. If alternatively, you are using a *small red non-recyclable sharps container* for biohazardous sharps, make sure that the lid is still closable when filled.

**How disposed of:**

- For the *small red biohazard* sharp container, close the lid of the filled sharps container and place it in a plastic bucket lined with an orange autoclave bag. Close the bag securely with the rubber bands provided with the bags. Autoclave and discard as regulated medical waste.

**Special note:**

Unbroken glass bottles or flasks are not considered sharps. They are collected in empty cardboard boxes. Once empty glass bottles have been collected, put the cardboard box in the hallway recognizable for the cleaning personnel that these are empty glass bottles for disposal.

## Biosafety Procedure 3

### Disposal of liquid infectious waste

#### Examples of waste:

Used ficoll tubes, blood collection tubes, flow cytometry tubes, liquid waste from vacuum traps, flow cytometry liquid waste.

#### How handled:

- Waste from vacuum traps or flow cytometry should have sufficient bleach (odor- and mercury-free) that the concentration is 1:10 is at all times. Whenever using the vacuum apparatus, you should aspirate 100% bleach to clean the line. Before emptying waste, add bleach so that the final solution is pale yellow. Allow to sit for 20 minutes before disposing.  
**Turn vacuum off** when not in use. Make sure that a **hydrophobic filter** is placed in the tubing between over-flow flask and vacuum house source (vacuum faucet) at all times.
- Ficoll tubes and blood collection tubes are tightly sealed and handled as solid infectious waste.
- *Fixed*, non-infectious liquid waste:  
Flow cytometry tubes containing fixed cells are tightly capped and disposed of in a puncture resistant, water tight, 5 gallon container.

#### How disposed of:

- Bring the trap in the bucket holding the trap to the sink. Discard bleached solutions of liquid waste down the drain of a “dirty” sink. Wear a face shield to avoid splashing into your face. It is advisable to run water after disposing of the bleached solution to hasten the removal of the bleach odor and to reduce the risk of corroding of the pipes. If the secondary overflow trap contains any liquid, empty this as well in the same manner. Before reattaching the tubings to the trap, pour sufficient 100% bleach onto the bottom of the main trap. Do not pour any bleach into the over-flow trap, because the bleach will lose activity
- Ficoll tubes and blood collection tubes are closed and disposed of as solid infectious waste. Avoid having too much liquid in the solid waste.
- Unautoclaved containers of flow cytometry tubes are placed in a double-lined (red bag) burn box and disposed of as regulated medical waste.



## **Biosafety Procedure 4**

### **Decontamination of biohazardous waste from BL2+ Laboratories**

#### **Waste affected:**

All waste containing or suspecting to contain etiologic agents infectious to humans.

- Any human or monkey blood (exposure risk examples: Hepatitis B or C, SIV, HIV, SHIV, and other bloodborne pathogens)
- Cultured cells or supernatants from cultured cells, known or suspected to contain HIV, SIV, or other bloodborne pathogens.

#### **Procedure:**

1. All waste will be placed in autoclavable orange biohazard bags and the bags contained within a hard plastic bucket. This bag will be closed with the rubber band provided with the bags (or tape). Waste will not overflow the top of the bucket.
2. Autoclave (250°F) should be closed tightly. Set it for 60 minutes sterilizing time and 30 minutes drying time in the “Dry cycle”. Press start button. Make sure the pressure in the chamber rises to 18-20 mm Hg, and stays there for 60 minutes. Pressure should fall to 0 before pressing off button and opening door. Crack the door for 10 minutes.

After decontamination, bags of waste will be placed in a double-lined (red bag), in a 4 cu. ft. cardboard box. When full, the lining bags will be individually sealed with tape, the box taped closed, and then placed in the hallway for pick up by Environmental Services. See Appendix C Boston College Biohazard Manual for more detailed information.

## **Biosafety Procedure 5**

### **Receiving blood samples of Human or non-Human Primate origin**

1. John O'Grady (Higgins operations 552-6778) or Brad Garman (Higgins operation assistant 552-0058) will be informed when we are expecting a shipment (by FedEx for national shipments or a courier service for local shipments).
2. Blood specimens will be delivered to Receiving on the first level.
3. Blood samples, contained in a biohazardous shipping container (see Appendix A), will be taken to the BL2 cell culture laboratory (457) and will remain locked until the experiment begins.
4. Within the clean area, the inner plastic container will be removed from the cardboard box. The cardboard box remains in the "clean" area.
5. The plastic container will be moved to a biosafety cabinet where it will be opened and blood tubes inspected for leakage or breakage. If all are intact, specimens will be distributed to investigators. If any leakage has occurred, it will be treated as a biohazardous spill and the lab director or biosafety officer notified before proceeding.
6. Assuming no leakage, the plastic container will be decontaminated with Cold Spore or similar disinfectant. Plastic container will then be washed with other glassware.
7. After it is washed the plastic inner container will be returned to the cardboard outer container and the package reused.
8. Shipping containers will be discarded when inner shipping container becomes contaminated with blood or when the outer box becomes wet, contaminated, or its integrity otherwise compromised.

## **Biosafety Procedure 6**

### **Action to take in case of accidental spill of biohazardous material**

1. Notify others in the area that a hazardous spill has occurred. Get help if needed. Attend to any injuries first
2. If necessary, evacuate the room for 20-30 minutes so that any aerosol generated will settle and not be inhaled.
3. **CONTAIN** and **DISINFECT**:  
Use the spill kit located in each lab.
  - SPILL KIT contents:**
    - concentrated bleach solution
    - heavy (utility)gloves and regular (medical examination) gloves
    - absorbent materials for liquids
    - paper towels
    - forceps, tweezers, or other mechanicals for handling sharps
    - small biohazard sharps container
    - biohazard bag for disposal
    - shoe covers
    - face protection (goggles and face mask)
    - (- possibly solid front gown)
    - (- possibly full face shield)
  - A. Small spills:  
Place paper towels over the spill to absorb it. Pour bleach on the paper towel and let it sit for at least 20 minutes before picking up the paper towel.
  - B. Large spills:  
Block off spill with paper towel to keep it from spreading. Carefully pour bleach onto the spill, let it sit, then pick up paper towels.
4. Use tongs to pick up sharps or broken glass. Never use your hands.
5. Dispose of all clean up materials as hazardous waste.
6. Inform laboratory biosafety officer that spill occurred and how it was cleaned up.
7. If you are unable to handle clean up yourself, call the EH&S (Sunil 2-0363).
8. If the spill has occurred in the BL2+ Sorting room, please refer to Biosafety Procedure 6A.

## **Biosafety Procedure 6A**

### **Emergency Procedure for BL2+ Sorting Room**

Follow all instruction that are outlined in Biosafety Procedure 6 (Action to Take in Case of Biohazardous Spill) and 11 (BL2+ Cell Sorting) to ensure your safety and that of others around you while containing the spill. If you have been exposed in the course of the accident, refer to Biosafety Procedure 8 (Emergency Procedures for Blood/Biohazard Exposure).

1. Immediately shut down the instrument to prevent the further spread of the spill/contamination.
2. Exit the room, follow the instructions provided in Biosafety Procedure 10 for removal of PPE, and notify all others in the area. Get help.
3. **DO NOT ENTER THE SORTER ROOM FOR AT LEAST 30 MINUTES AFTER THE SPILL.** This will allow most of the aerosols to settle.
4. **YOU MUST WEAR THE ENTIRE BIOSAFETY SUIT TO DECONTAMINATE THE ROOM.** It is advisable that there be at least one other person in the sorter room to help with the decontamination. Do not try to handle all of this on your own.
5. All porous surfaces that cannot be easily disinfected (i.e., ceiling tiles, pipe insulation etc.) must be removed, soaked in bleach, secured in a large orange autoclave bag and placed on a cart in one of the tissue cultures labs designated for items to be autoclaved.
6. All other surfaces must be decontaminated according to Biosafety Procedure 10. This includes the floors, walls, all furniture, the instruments and cabinets.

If you are unable to handle clean up yourself, call EH&S (Sunil 2-0363).

## **Biosafety Procedure 7**

### **Shipping of dangerous goods**

For shipping of dangerous goods a **special training** is required. This training is provided through the biosafety officer (Eh&S Sunil 2-0363). If you want to have dangerous goods sent to another institution, please inform the person who has this special training ahead of time so that the necessary paperwork can be filled out.

Examples of **dangerous goods** include shipping on dry ice and shipping of human or non-human primate blood or frozen plasma.

Packing blood for shipment:

1. All blood drawn will be drawn in or transferred to vacutainer tubes. Heparinized blood will be drawn into heparinized syringes and transferred into red-topped clot tubes (without paraffin). Nothing is ever shipped with needles attached.
2. Filled blood tubes will be placed in a sealable biohazard plastic bag with several gauze sponges, bag sealed and placed in the inside plastic container of the SAF-T-PAK biohazard shippers. The plastic container will be closed tightly (see Appendix A).
3. The plastic container will be placed inside the cardboard shipping box and the top of the box secured. (These boxes are reused so excessive taping is not required.)
4. The package will be delivered to the receiving dock along with a hazardous materials declaration for pickup by the scheduled courier. The forms are already made out but should be signed and dated.
5. The courier is always instructed to arrive after 11:00 am for pickup.

#### **Biohazard shipment**

For shipment of infectious substances (e.g. viral stocks) special regulations apply and extra paperwork is required as per Fed Ex or World Courier and USDA requirements. Please contact the person shipping well ahead of time to make sure that all regulations are met.

## **Biosafety Procedure 8**

### **Emergency Procedures for Blood/Biohazard Exposure**

(Adapted from *Blood/Body Fluid Exposure Information, BIDMC Infection Control Department and Employee/Occupational Health Services Guidelines IC-BBP4 and IC-BBP6, and Holmes GP, Chapman LE, Stewart JA, Straus SE, Hilliard JK, Davenport DS. Guidelines for the prevention and treatment of B-virus infections in exposed persons. Clin Infect Dis 1995; 20:421-39.*)

#### **Definitions:**

##### **Blood/Biohazard-**

- Any human or monkey blood (exposure risk: Hepatitis B, Hepatitis C, HIV, SIV, SHIV, Herpesvirus simiae also known as B-virus)
- Cultured cells or supernatants from cultured cells, known or suspected to contain HIV, SIV, SHIV, or other bloodborne pathogens

##### **Exposure-**

Where any of the above blood/biohazards are involved in:

- Needlestick or puncture accident with sharp, contaminated equipment.
- Mucous membrane exposure (e.g. splash to eye or mouth)
- Cutaneous exposure (e.g contact with ungloved hands or other skin surface that may be cut, chapped, abraded or affected by active dermatitis)

#### **1. FIRST AID**

What you should do if exposed:

- A. Force bleed the site if possible.
- B. Clean wound with soap and water (special products are not necessary).
- C. Apply direct pressure if needed to stop bleeding.
- D. If eye/mucous membrane is exposed, flush with water from eyewash for 15 minutes.

#### **2. After first aid:**

- A. Prompt notification is important to evaluate possible treatment options. Time is very critical – if post exposure prophylaxis is deemed necessary, it should be started within minutes to a few hours.

**\*\*\*Inform the health care provider that the CDC advises that post-exposure prophylaxis for SIV should be handled in exactly the same way as HIV exposure**, as outlined in “Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HIV and Recommendations for Postexposure Prophylaxis” MMWR Vol 54, No RR9;1, September 2005 (Appendix B).

Employees should follow the guidelines outlined in the “Boston College Bloodborne Pathogens Exposure Control Plan.” (Appendix C)

B. “Due to the need for urgent initiation of treatment, which should be initiated within 2 hours as recommended by current HIV prophylaxis protocols, the employee will be offered post exposure HIV (SIV) evaluation and prophylaxis through immediate referrals to St. Elizabeth’s Occupational Health Department.”

## **Biosafety Procedure 9**

### **Interlaboratory Transfer of Cell Lines**

Cell lines that have been maintained in laboratories that propagate viruses or other organisms have the potential to unknowingly harbor etiologic agents.

#### **General Practices**

1. Whenever possible, obtain cell lines from a repository or verified source, such as American Type Culture Collection or the NIH AIDS Research and Reference Reagent Program.
2. Always treat cell lines as a biological hazard, regardless of the source of the line.
3. Avoid transferring cell lines out of the laboratory unless absolutely necessary.

#### **Specific Practices**

1. For cell lines entering the laboratory *from sources other than repositories*, treat as biohazardous and culture at BSL2 or greater.
2. When transferring cell lines to other laboratories, include a written description of the material. In addition, note *in writing* that the cell line was maintained in a BSL2+ laboratory where pathogenic viruses were propagated. Recommend handling this material as biohazardous.
3. File a copy of this letter in the department office.

## Biosafety Procedure 10

### BL2+ Cell Sorting

All BL2+ procedures must be followed.

1. In preparation for a biohazardous sort the operator must first prepare the cell sorter as follows:
  - A. Empty the waste tank and add enough bleach that the final concentration of bleach will be 10%.
  - B. Fill the sheath supply tank with adequate sheath fluid.
  - C. Confirm that there is adequate dH<sub>2</sub>O in the cytometer rinse bottle.
  - D. Turn on the sorter and confirm proper optical alignment.
  - E. Adjust the video camera and U.V. microscope for proper viewing. (This needs to be used during sort set-up and actual sorting.)
  - F. Adjust the cell sorting functions using appropriate microbeads.
  
2. The following must be available in the sorter room prior to a biohazardous sort:
  - A. Ethanol-70% solution
  - B. Bleach-10% solution
  - C. dH<sub>2</sub>O
  - D. Biohazard buckets and biohazard bags (enough for the sort)
  - E. Paper towels (at least 1 package)
  - F. Extra gloves (may be kept in the cabinet within the sorter room)
  - G. Extra blue gown (may be kept in the cabinet within the sorter room)
  - H. Ice (if necessary)
  - I. A spare battery pack.
  - J. A complete phone list including the way in which to reach the investigator.
  
3. When all items have been assembled and the sorter prepared, the operator will get dressed in the biohazard outfit--outside the sorter room. The personal protective equipment must be worn in the following order:
  - A. Coverall zipped to the collar.
  - B. Boots over the operator's shoes and coverall to the knees.
  - C. Belt with fully charged batteries & filters adjusted so the operator can sit comfortably.
  - D. One pair of long gloves over the cuffs of the coveralls.
  - E. Helmet with a new hood.
  - F. Blue gown tied with the opening in the back.
  - G. A second pair of gloves over the cuffs of the blue gown.
  
4. The operator will then place a sign on the sorter room door indicating that there is a biohazardous sort in progress. The operator will then enter the sorter room with the specimen(s) to be sorted and close the door.
  
5. Initial Decontamination of the Sorter Room:
  - A. Using gauze soaked with 10% bleach solution, wipe the inside of the sample introduction area, the sort collection area and the counter top.
  - B. Using gauze soaked in distilled water, rinse the areas that were cleaned with bleach.
  - C. Spray down the entire outside of the instrument, computer, counter top, floor, chairs and any other piece of furniture/equipment present in the room with enough 70% ethanol to cover.
  - D. You **must** wait a minimum of 15 minutes from the end of the sort before the door to the sorter room is opened. This allows some of the aerosols to settle.
  - E. Once outside the room, the operator must post a sign on the door stating that a sort was just



completed and that no one should enter the sorter room.

F. **DO NOT EMPTY THE WASTE CONTAINER IMMEDIATELY AFTER SORTING.**

6. Removal of Personal Protective Equipment:

- A. Remove the outer pair of gloves before exiting the sorter room and discard in a biohazard bucket that is lined with an orange autoclave bag.
- B. Remove the blue gown, boot covers, hood, coverall and gloves and place them in the biohazard box that is lined with a large orange autoclave bag. This **must** be done outside of the sorter room.
- C. Wipe down the helmet with gauze soaked in 70% ethanol.

7. Decontamination of the Sorter Room 12 Hours Post Sort:

- A. Enter the room wearing a blue gown and gloves (standard BL2 procedures).
- B. Spray down all surfaces with 70% ethanol then wipe the surfaces using gauze or paper towels. Discard the gauze/paper towels in a biohazard bucket lined with a small orange autoclave bag. Secure the bag closed using a rubber band.
- C. Discard any remaining sample tubes in a sharps container.
- D. Discard and replace the biohazard filter. Place the "dirty" filter in a biohazard bucket lined with a small orange autoclave bag.
- E. Secure closed any remaining orange, autoclave bags using a rubber band.
- F. Bring the biohazard buckets to one of the tissue culture labs and place it on a cart that is designated for items to be autoclaved.

**THE WASTE CONTAINER MAY NOW BE EMPTIED INTO A SINK THAT IS DESIGNATED FOR LIQUID WASTE.**

**For reference see Appendix D.**



## **Biosafety Procedure 11**

### **Centrifuge usage**

Tubes with human or rhesus blood, serum or other bodily fluids must only be opened in the biosafety cabinet (Higgins 457).

1. Bring the centrifuge adaptors, buckets and sealing caps to the biosafety cabinet. Put your tubes in the centrifuge buckets with adaptors to spin, attach the sealing cap, and only then remove the entire sealed bucket from the biosafety cabinet into the centrifuge.
2. After the spin is completed, remove the entire sealed bucket from the centrifuge into the biosafety cabinet and then remove the sealing cap and samples.
3. Inspect for spills in the buckets (if there was leakage or a spill in the centrifuge buckets, refer to Biosafety Procedure 6 for decontamination.)
4. Before you remove the buckets from the hood, reattach the sealing caps.