



FOOD SAFETY LAB / MILK QUALITY  
IMPROVEMENT PROGRAM



*Standard Operating Procedure*

Title: General safety procedures and handling and disposal of laboratory waste, including biohazard materials

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***General safety procedures and handling and disposal of laboratory waste, including biohazard materials***

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## SECTION 1 INTRODUCTION

### 1.1 Purpose

The purpose of this document is to set forth guidelines for handling BSL-1 and BSL-2 materials and other waste.

In addition, subsections in this document describe specific procedures for handling BSL-2 solid and liquid wastes generated from all APHIS permit-related work.

### 1.2 Scope

This SOP applies to anyone working in Stocking Hall rooms 350-358 (Food Safety Laboratory, MQIP Laboratory, Laboratory For Molecular Typing, Alcaine Laboratory, Snyder Laboratory, and Worobo Laboratory) and any experimental procedures conducted by laboratory members at other locations.

### 1.3 Definitions

#### 1. BSL-1 and BSL-2:

**(1a) BSL-1** organisms are not normally pathogens; their risk to the worker is small. They may be *E. coli* K12 strains used in routine cloning, or they may be benign species such as *L. innocua*.

#### **Biosafety Level (BSL) 1 Organisms commonly used in the Food Safety Laboratory:**

- *Aeromonas hydrophilia*
- *Bacillus thuringiensis*
- Other non-pathogenic *Bacillus* spp.
- Non-pathogenic *Clostridium* spp
- Non-pathogenic *Escherichia coli*
- *Listeria innocua*, *Listeria seeligeri*, *Listeria welshimeri*, etc.
- *Pseudomonas* spp (except *aeruginosa*)
- *Streptococcus uberis*

**(1b) BSL-2** organisms are infectious to humans (and sometimes to animals) and BSL-2 waste is considered regulated medical waste. The risk to the worker is higher than with BSL-1. Organisms such as *Listeria monocytogenes* are considered BSL-2.

#### **Biosafety Level (BSL) 2 Organisms commonly used in the Food Safety Laboratory:**

- *Bacillus cereus*
- *Listeria monocytogenes*



- *Salmonella enterica* including *Typhi*
- *Escherichia coli* 0157 and other Shiga toxin producing *E. coli* from field studies
- *Mycobacterium avium* subspecies *paratuberculosis* (storage only)
- *Vibrio parahaemolyticus* (storage only)
- *Rhodococcus equi*
- any human cell lines, and any animal (non-primate) cell lines that are:
  - Obtained from an outside source (e.g. ATCC) or,
  - Established within a lab without a complete history or,
  - Have been contaminated at any time with a zoonotic agent or,
  - Derived from genetically manipulated cells capable of supporting the replication of infectious human and zoonotic agents or,
  - Were previously exposed to a virus containing recombinant DNA or RNA, including recombinants from non-zoonotic viruses.

## 2. Laboratory waste- Reusable glassware, including

(2a) Glassware that has not been in contact with biological materials

(2b) **BSL-1 glassware:** Glassware that has been in contact with BSL-1 biological materials

(2c) **BSL-2 glassware:** Glassware that has been in contact with BSL-2 biological materials or other materials that should be classified as BSL-2 (i.e., regulated medical waste)

## 3. Single use disposable glass culture tubes, including

3a) Tubes that have not been in contact with biological materials

3b) **BSL-1 tubes:** Tubes that have been in contact with BSL-1 biological materials

3c) **BSL-2 tubes:** Tubes that have been in contact with BSL-2 biological materials or other materials that should be classified as BSL-2 (i.e., regulated medical waste)

## 4. Single use materials

(4a) **BSL-1 trash:** Single use materials visually identifiable as laboratory materials (e.g., pipet tips, etc.) which have not been in contact with biological materials or which have been in contact with BSL-1 biological materials

(4b) **BSL-2 trash:** Single use materials that have been in contact with BSL-2 biological materials or other materials that should be classified as BSL-2 (i.e., regulated medical waste)

## 5. Sharps

Sharps are defined in the "National Guidelines for the Management of Clinical and related Wastes" published by the National Health and Medical Research Council as "objects or devices having acute rigid corners, edges, points or protuberances capable of cutting or penetrating the skin". Hypodermic needles, Pasteur pipettes, and scalpel blades all fit this definition.



**6. APHIS permit (Animal and Plant Health Inspection Service Plant Protection & Quarantine permit, USDA)**

A USDA permit to move live plant pests, noxious weeds, and soil across different States in the US. The lab current APHIS permit #P526P-21-01561, is valid through 04/22/2024.



## 1.4 Safety

This section has been left blank, as this entire document describes laboratory safety.

## SECTION 2 MATERIALS

- 20% chlorine bleach
- 10% Lysol
- 70% ethanol
- Disposable gloves
- Clear autoclave bags
- Red Biohazard bags

## SECTION 3 PROCEDURES

### 3.1 General Laboratory Safety Procedures

- No eating or drinking in the laboratory.
- Proper PPE (glove, lab coats and safety glasses) must be worn when working in the lab.
- Wash your hands after removing your gloves and before leaving the laboratory.
- Do not wear your PPE outside of the laboratory.
- Never Re-use gloves.
- Disinfect benchtops and pipettors with 70% ethanol, 10% bleach or Lysol before and after use with BSL-1 or BSL-2 organisms.
- Liquid BSL-2 waste is to be treated with chlorine bleach to a final concentration of 10% for 30 minutes at the (BSL-2) treatment center in room 352 by the individual who generated the waste.

### 3.2. Separation and treatment of glassware

- (1) Reusable laboratory glassware is separated into two categories
  - Glassware that has not been in contact with biological materials
  - **BSL-1 and BSL 2 glassware:** Glassware that has been in contact with BSL-1 or BSL 2 biological materials.
- (2) Single-use glass culture tubes that are disposable (except caps) are separated into 3 categories.
  - Disposable Glass Culture Tubes that have not been in contact with biological materials.



- Disposable Glass Culture Tubes that have been in contact with BSL-1 biological materials.
- Disposable Glass Culture Tubes that have been in contact with BSL-2 biological materials.

Glass should NEVER be disposed of in a regular BSL-1 or BSL-2 waste bin!

**Treatment of reusable glassware that has not been in contact with biological materials:**

Glassware that has not been in contact with biological materials is to be placed in bins that are labeled “dirty glassware”. Glassware located in these bins will be washed without prior autoclaving. There is a bin in each of the following rooms in the lab; Rooms 350, 352, 354, 356.

**Treatment of BSL-1 and BSL-2 reusable glassware**

Glassware that has been in contact with BSL-1&2 biological materials is to be treated with chlorine bleach at the designated BSL-1&2 liquid waste treatment station in the lab (currently in room Stocking Hall 352 Autoclave Room); an equal volume of 20% bleach has to be added to any liquid waste, followed by a treatment period of 30 minutes. The treated waste can then be disposed in the drain and the glassware rinsed and filled with water. The treated glassware is autoclaved and then washed.

**Treatment of disposable glass culture tubes that have NOT been in contact with biological materials:**

Place tubes in glassware collection bins located in Rooms 350, 352, 354, and 356.

**Treatment of BSL-1 disposable glass culture tubes**

Place racks of culture tubes in Room 352, autoclave room, waste treatment area in the bin labeled BSL-1 Glass Culture Tubes. Media Room Technicians will autoclave the tubes, place them in a plastic bag inside a cardboard box, and dispose of them in regular trash.

**Treatment of BSL-2 disposable glass culture tubes**

Place racks of culture tubes in Room 352, autoclave room, in the bin labeled BSL-2 Glass Culture Tubes. Media Room Technicians will autoclave the tubes, and place them in a Sharps container, which will be picked up by EHS.

**Treatment of BSL-2 disposable glass culture tubes generated by projects covered by the APHIS permit**

An equal volume of 20% bleach is added to the culture tubes, followed by a treatment period of 30 minutes. The treated waste can then be disposed of in the drain. Media Room Technicians will autoclave the tubes and place them in a Sharps container, which EHS will pick up.



### 3.3. Separation and treatment of waste generated by projects covered by the APHIS permit

#### 3.3.1. Treatment of BSL-1 solid and liquid wastes

Solid and liquid BSL-1 wastes are to be separated and treated using the same general procedures described in this document.

#### 3.3.2. Treatment of BSL-2 solid and liquid wastes

##### Treatment of all solid materials that have been in contact with BSL-2 biological materials

All solid wastes generated by projects in the framework of the APHIS permit and have been in contact with BSL-2 biological materials must be processed as **Regulated Medical Waste**. This includes shipping boxes and inner shipping materials such as foam boxes, plastic bags, absorbents etc.,. BSL-2 solid waste is placed in double clear bags, placed inside a red Biohazard bag (hence triple bagged), tagged, and picked up by Environmental Health and Safety to be autoclaved in a certified autoclave.

##### Treatment of all liquid materials that have been in contact with BSL-2 biological materials

All liquid wastes generated in the framework of the APHIS permit and have been in contact with BSL-2 biological materials must be treated with chlorine bleach to a final concentration of 10% for 30 minutes. ***This includes liquid cultures in disposable glass tubes.***

### 3.4. BSL-1 and BSL-2 Waste Disposal

Single use materials that are not glass and are not washed and re-used are separated into two categories:

- (1) **BSL-1 waste:** Single use materials visually identifiable as laboratory materials (e.g., pipet tips, etc.) which have not been in contact with biological materials or which have been in contact with BSL 1 biological materials
- (2) **BSL-2 waste:** Single use materials that have been in contact with BSL-2 biological materials or other materials that should be classified as BSL-2 (i.e., regulated medical waste)

BSL-1 is autoclaved in the laboratory then placed in garbage bags before being disposed of in the trash dumpster.

BSL-2 is contained in double clear bags, placed inside red Biohazard bags (hence triple bagged), tagged, and picked up by Environmental Health and Safety.

PPE must be worn while collecting, bagging or portioning BSL-1 or BSL-2 waste!





### Autoclaving BSL-1 waste

- All waste must be in double clear bags.
- Autoclave bags must be clearly labeled as BSL-1 and require a piece of autoclave tape.
- Do not overfill autoclave bags. ~5/8 full is a good estimate. If a bag is too heavy, please portion into a separate bag. Overfilling bags may result in incomplete sterilization of the contents.
- BSL-1 is autoclaved for 60 minutes at 121° C, allowed to cool, then placed in plastic garbage bags.

### **3.5. Sharps Disposal**

Sharps are not further separated. All sharps (regardless of whether they have not been in contact with any biological materials or whether they have been in contact with BSL-1 or BSL-2 materials) are to be placed in the red sharps containers, which are found throughout the labs. Do not fill the containers past the “Full” line.

When full, they are taped shut and tagged for pick-up by Environmental Health and Safety.

### **3.5 Serological Pipette (Stripette) Disposal**

Serological pipettes are not further separated. ALL serological pipets are to be placed in the small, cylindrical, double-bagged waste containers located throughout the lab, regardless of level of biological contamination. When full they are placed in red Biohazard bags and tagged for pick-up by Environmental Health and Safety.

### **3.6 Liquid Enrichments in Sample Bags.**

Samples that have not been in contact with biological material may be emptied in the sink in the Autoclave Room and the bags disposed of in regular trash.

Samples that have been in contact with BSL-1 or BSL 2 Biological materials are to be left in the bags and disinfected by adding bleach to reach a final concentration of 10%. After a dwell time of 30 minutes, the bleached enrichment can then be drain disposed and the enrichment bags placed in a BSL2 bin for collection by EH&S. Do not put full enrichment bags directly into BSL 2 containers. They leak, and EH&S will not pick them up.

### **3.7 Reusable Glassware that contains antibiotics or substances that cannot be mixed with bleach.**

- ALL antibiotic solutions regardless of level of biological contamination must be autoclaved before disposal down the sink. If your waste glassware contains an antibiotic, make sure that it is clearly marked.
- ALL glassware that has been in contact with BSL-1 or BSL-2 substances and contains chemicals that react adversely with bleach (paracetic acid, phenol chloroform, etc) must be clearly labeled when placed on the treatment bench. Do not add bleach. Just mark the



containers so that Media Room personnel know to not add bleach.



### **3.8 Soil samples, labware, and packaging material covered under APHIS permit P526P-21-01561**

- All soil samples acquired for this project must be treated as *Regulated Medical Waste*.
- All disposable labware that has come in contact with the soil samples must be disposed of as BSL-2 waste.
- Any reusable labware that has come in contact with the soil samples (beakers, spatulas, etc.) must be either treated with a 10% bleach solution for a 30-minute dwell time and autoclaved at 121° C for 30 minutes prior to washing.
- Soil primary packaging material should be treated as BSL-2 waste.

### **3.9 Labeling**

Within the lab, all waste containers are labeled either BSL-1 or BSL-2. When collected, each bag of BSL-2 waste is tagged with a pre-made label. The waste is then placed in the large red EH&S Biohazard trash containers in room 358 to await pick-up by Environmental Health and Safety. Pickup is typically once a week on Thursday. If too many bags are accumulating, call EH&S to pick up a second time.

### **3.10 Biological Spills**

Small spills that are easily contained may be cleaned up with the laboratory spill kit located in 352D.

Refer to Appendix C of the Biological Safety Manual “What to do in the Event of a Biohazardous Material Spill”, or call EH&S if you are unsure how to proceed

## **SECTION 4 TROUBLESHOOTING**

To report any situation that requires IMMEDIATE police, fire, or medical response to preserve life or property:

- Dial 911 from the laboratory phone
- Dial 607-255-1111 from a cell phone
- Use the emergency phone located on the hallway wall outside of Room 350

If you have a question or concern that cannot be answered by laboratory personnel, contact Cornell EH&S either online (<https://ehs.cornell.edu/about-us/contact-us/askehs>) or by phone (607-255-8200).

## **SECTION 5 REFERENCES**

<http://www.ehs.cornell.edu/> Guidelines for the Disposal of Regulated Medical Waste



Biological Safety Levels 1 and 2 Manual.

**SECTION 6 METHOD VERSION & CHANGES**

| <b>VERSION</b> | <b>DATE</b> | <b>EDITOR</b>  | <b>COMMENTS</b>  |
|----------------|-------------|----------------|--|
| Version 0      | 2004-04-15  | nhw6 and bmb29 | Original SOP   |
| Version 1      | 2016-06-27  | ser15          | Original SOP version posted on Wiki                      |
| Version 2      | 2017-08-23  | nrs13          |  |
| Version 3      | 2018-02-13  | mgg53 and vg93 | Updated with new EH&S recommendations for waste handling |
| Version 4      | 2021-02-17  | ser15          | Converted to new SOP format with updated information     |
| Version 5      | 2022-04-26  | ag67           | Updating APHIS-related information                       |
| Version 6      |             |                |  |
| Version 7      |             |                |  |