



**FOOD SAFETY LABORATORY  
CORNELL UNIVERSITY**

**General safety, handling and disposal procedures of  
biological laboratory waste (Including BSL-1 organisms  
and BSL-2 pathogens)**

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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 the Food Safety Laboratory.

### 1.2 Scope

This SOP applies to anyone working in the Food Safety Lab (FSL), Milk Quality Improvement Program (MQIP), and Laboratory for Molecular Typing (LMT) 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 entérica* 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- Re-usable 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.



## SECTION 2 MATERIALS

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

## SECTION 3 SAFETY AND WASTE DISPOSAL PROCEDURES

### 3.1 General Laboratory Safety Procedures

- No eating or drinking in the laboratory.
- Always wear gloves when handling BSL-2 organisms. Wash hands after handling BSL-1 or BSL-2 materials (even though you were wearing gloves).
- Never Re-use gloves.
- Wipe down benchtop & pipettors with 70% ethanol, 10% bleach or Lysol before and after use with BSL-1 or BSL-2 organisms.
- Use gloves for ANY centrifugation and aspiration since BSL-1 and BSL-2 materials share the same centrifuge.
- All liquid BSL-2 waste is to be treated with chlorine bleach to a final concentration of 10% at the (BSL-2) treatment center in room 352 by the individual that generated the waste.

### 3.2. Separation and treatment of glassware

(1) Re-usable 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 re-usable 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 re-usable 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 35, 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.

**3.3. 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.

Gloves 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 will result in less than optimal autoclaving.
- BSL-1 is autoclaved for 60 minutes at 121° C, allowed to cool, then placed in black plastic garbage bags.

#### **3.4. 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. 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 the sample bag containers placed on the BSL waste treatment counter. Media room technicians will empty the liquid from the bags into a graduated container. Bleach will be added to the container to reach a final minimum concentration of 10% bleach. The bleached enrichment will then be poured into the sink and the enrichment bags will be placed in BSL-2 bins for collection by EH&S. Do not put enrichment bags directly into BSL 2 containers. They leak, and EH&S will not pick them up.

#### **3.7 Re-usable Glassware that contain 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.



#### **SECTION 4            REPORTING and 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.

#### **SECTION 5            TROUBLESHOOTING**

Not applicable

#### **SECTION 6            REFERENCES**

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