



**FOOD SAFETY LABORATORY
CORNELL UNIVERSITY**

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

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TABLE OF CONTENTS

1.	INTRODUCTION	3
	1.1 Purpose	
	1.2 Scope	
	1.3 Definitions	
	1.4 Safety	
2.	MATERIALS	4
3.	PROCEDURE	6
4.	REPORTING AND LABELING	8
5.	TROUBLESHOOTING	10
6.	REFERENCES	11



SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth guidelines for handling BSL-1 and BSL-2 materials (also known as P1 and P2 within the lab) and other waste in the Food Safety Laboratory.

1.2 Scope

This SOP applies to the Food Safety Lab, including the Laboratory for Food Microbiology and Pathogenesis of Foodborne Diseases 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*.

Commonly used 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*, which must be ingested in large numbers to sicken anyone, 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-intracellulare*
- *Mycobacterium paratuberculosis*
- *Vibrio parahaemolyticus*
- *Rhodococcus equi*
- human blood, blood products, or pathological wastes
- any animal blood, blood products, or pathological wastes, bedding, and carcasses
- 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 materials

(3a) 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

(3b) 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)

4. 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

I. 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).
- 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. Do not forget your waste!

II. Separation and treatment of glassware

Re-usable laboratory glassware is separated into three categories

- (1a) Glassware that has not been in contact with biological materials
- (1b) **BSL-1 glassware:** Glassware that has been in contact with BSL-1 biological materials
- (1c) **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)

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

Treatment of 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.

Treatment of BSL-1 glassware

Glassware that has been in contact with BSL-1 biological materials is to be placed in bins labeled BSL-1 glassware. BSL-1 glassware will be autoclaved and subsequently be washed.

Treatment of BSL-2 glassware

Glassware that has been in contact with BSL-2 biological materials is to be treated with chlorine bleach at the designated BSL-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.



III. BSL-1 and BSL-2 Waste Disposal

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

(2a) **BSL-1 waste:** Single use materials visually identifiable as laboratory materials (e.g., pipett tips etc.) which have not been in contact with biological materials or which have been in contact with BSL 1 biological materials

(2b) **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 black 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 (or P1) 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.

IV. 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.



SECTION 4 REPORTING and LABELING

Within the lab, all waste containers are labeled either BSL-1 or BSL. When collected, all BSL-2 waste is tagged with the name of the technician supervising waste disposal that month, the lab, department, phone number and date. The waste is then placed in red Biohazard trash containers to await pick-up by Environmental Health and Safety. Pickup is typically twice a week.

SECTION 5 TROUBLESHOOTING

Not applicable

SECTION 6 REFERENCES

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