

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
Page #: Page 1 of 14	Last Reviewed/Update Date: November 2015
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SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

Biological Safety Level 1 (“BSL1”) and Biological Safety Level 2 (“BSL2”) Biological Waste Management Guideline

1.0 Purpose and Scope

The purpose of this Guideline is to describe the procedures for handling, disposing and destroying medical and/or biological waste involving Risk Group 1 (“RG1”) and Risk Group 2 (“RG2”) agents generated in *BSL1 and BSL2* research, training and clinical laboratories (hereinafter referred to as BSL1 and BSL2 laboratories).

This Guideline is developed to assist personnel who utilize microbiological laboratories at the Boston University Charles River Campus (“CRC”) in the proper disposal of biological waste generated by their operations. This Guideline establishes the minimum requirements for RG1 and RG2 biological waste management in *BSL1 and BSL2 laboratories*.

2.0 References

2.1 Regulations and Guidelines

- 2.1.1 105 CMR 480 State Sanitary Code – Commonwealth of Massachusetts
- 2.1.2 29 CFR 1910.1030 Bloodborne Pathogens Standard – Occupational Safety and Health Administration (“OSHA”)
- 2.1.3 Biosafety in Microbiological and Biomedical Laboratories (“BMBL”) 5th edition – Centers for Disease Control and Prevention
- 2.1.4 NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (November 2013)– National Institutes of Health
- 2.1.5

2.2 Supplementary Documents

Boston University Biosafety Manual

3.0 Definitions

3.1 Biological Waste – The following are defined as medical or biological waste, per 105 CMR 480.000 and the applicable federal regulations and guidelines::

- 3.1.1 *Blood and Blood Products*: Discarded bulk human blood and blood products in free draining, liquid state; body fluids contaminated with

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
	Last Reviewed/Update Date: November 2015
Page #: Page 2 of 14	Approval Authority: IBC
Expiration Date:	
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

visible blood; and materials saturated/dripping with blood. Blood and Blood Products shall not include: feminine hygiene products.

3.1.2 *Pathological Waste:* Human anatomical parts, organs, tissues and body fluids removed and discarded during surgery, autopsy, or other medical or diagnostic procedures; specimens of body fluids and their containers; and discarded material saturated with body fluids other than urine.

Pathological waste shall not include: Teeth and contiguous structures of bone without visible tissue, nasal secretions, sweat, sputum, vomit, urine, or fecal materials that do not contain visible blood or involve confirmed diagnosis of infectious disease.

3.1.3 *Cultures and Stocks of RG1 or RG2 Infectious Agents and their Associated Biologicals:* All discarded cultures and stocks of infectious agents and their associated biologicals, including culture dishes and devices used to transfer, inoculate, and mix cultures, as well as discarded live and attenuated vaccines intended for human use, that are generated in:

3.1.3.1 Laboratories involved in basic and applied research;

3.1.3.2 Laboratories intended for educational instruction; or

3.1.3.3 Clinical laboratories

3.1.4 *Contaminated Animal Waste:* Contaminated carcasses, body parts, body fluids, blood or bedding from animals known to be:

3.1.4.1 Affected with, or suspected to be affected with, a contagious disease or any suspicion or occurrence of any such disease if it is potentially infectious to humans that are reportable to the Massachusetts Department of Agricultural Resources, Bureau of Animal Health pursuant to 105 CMR 300.140:

3.1.4.1.1 ; or

3.1.4.2 Infected with diseases designated by the State Epidemiologist and the State Public Health Veterinarian as presenting a risk to human health; or

3.1.4.3 Inoculated with infectious agents for purposes including, but not limited to, the production of biologicals or pharmaceutical testing; or

3.1.4.4 Altered via recombinant or synthetic nucleic acid molecules; including transgenic animals and animals which have been exposed to recombinant or synthetic nucleic acid-altered micro-organisms.

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
	Last Reviewed/Update Date: November 2015
Page #: Page 3 of 14	Approval Authority: IBC
Expiration Date:	
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

3.1.5 *Sharps*: Discarded medical articles that may cause puncture or cuts, including, but not limited to, all needles, syringes, lancets, pen needles, pasteur pipettes, broken medical glassware/plasticware, scalpel blades, suture needles, dental wires, and disposable razors used in connection with a medical procedure.

3.1.6 *Biotechnology By-Product Effluents*: Any discarded preparations, liquids, cultures, contaminated solutions made from microorganisms and their products including genetically altered living microorganisms and their products. This definition includes recombinant nucleic acid molecules, synthetic nucleic acid molecules and any cells, organisms or viruses containing such molecules.

3.2 RG1 Microorganism - RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include, but are not limited to: asporogenic *Bacillus subtilis* or *Bacillus licheniformis*; adeno- associated virus (“AAV”)(all serotypes); recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus; and strain of *Escherichia coli* is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (*i.e.*, lacks the O antigen) and (2) does not carry any active virulence factor (*e.g.*, toxins) or colonization factors and does not carry any genes encoding these factors.

3.3 RG2 Microorganism: RG2 agents are associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available. See NIH Guideline (http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html#_Toc351276293) list of RG2 organisms.

4.0 Roles & Responsibilities

4.1 Laboratory Staff manages biological waste within the BSL1 and BSL2 laboratories per the BU Biosafety Manual requirements. Laboratory Staff:

4.1.1 Manages supplies, biohazard bags, biohazard boxes, sharps containers, autoclave bags and disinfectant within the laboratory,

4.1.2 Collects biological waste in suitable waste containers,

4.1.3 Segregates biological waste from other wastes,

4.1.4 Notifies EHS when full boxes are generated using the online pickup request form,

4.1.5 Chemically disinfects liquids,

4.1.6 Loads, operates and unloads the autoclave if applicable,

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
Page #: Page 4 of 14	Last Reviewed/Update Date: November 2015
Expiration Date:	Approval Authority: IBC
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

- 4.1.7 Packages biological waste in a double-bagged biohazard waste box and/or sharps container in the laboratory,
 - 4.1.8 Attaches any required labels to the box, and
 - 4.1.9 Maintains the laboratory record-keeping log for autoclaved biological waste.
- 4.2 Environmental Health and Safety (“EHS”) supports the Laboratory Staff through collection of full containers, record keeping, training, auditing of procedures, vendor selection/audit and emergency response. EHS:
- 4.2.1 Provides Biological Waste training to all Laboratory Staff conducting work in BSL1 and BSL2 laboratories. Biological waste training is included in the annual Laboratory Safety Training.
 - 4.2.2 Provides for the pickup and staging of full boxes of biological wastes from CRC laboratories,
 - 4.2.3 Participates in the vetting and selection of biological waste disposal vendors,
 - 4.2.4 Manages the paperwork involved with shipping biological waste,
 - 4.2.5 Maintains the record-keeping log for all medical or biological waste shipped off-site for treatment. The log includes:
 - (a) The exact date of each shipment;
 - (b) The total number of containers;
 - (c) The type of waste;
 - (d) The total combined weight or volume;
 - (e) The name of the transporter with shipping identification number (if applicable)
 - 4.2.6 Reviews and approves biological waste storage rooms/locations,
 - 4.2.7 Maintains a current listing of approved storage rooms/locations (*Appendix A*),
 - 4.2.8 Reviews and approves the use of autoclaves for the sterilization of BSL1 and BSL2 waste,
 - 4.2.9 Maintains a current listing of approved autoclaves (*Appendix B*),
 - 4.2.10 Provides bound Record-Keeping Log books where required by this procedure,
 - 4.2.11 Verifies that shipping papers generated are matched with corresponding medical waste tracking forms for each shipment,
 - 4.2.12 Audits Laboratory Staff procedures and operations to assure compliance with procedures and regulatory requirements, and
 - 4.2.13 Provides clear instructions to generators of biological waste through training and on the EHS web site.

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
Page #: Page 5 of 14	Last Reviewed/Update Date: November 2015
Expiration Date:	Approval Authority: IBC
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

4.3 Biohazardous Waste Contractor

- 4.3.1 Provides pickup from designated storage locations
- 4.3.2 Provides boxes, bags, labels and tape for biological waste collection
- 4.3.3 Brings biological waste through to final destruction offsite
- 4.3.4 Completes destruction paperwork and notifies EHS of discrepancies

4.4 Institutional Biosafety Committee (IBC)

- 4.4.1 Performs annual review of this waste policy
- 4.4.2 Reviews and approves the waste disposal plan in individual IBC protocol
- 4.4.3 Proposes enhanced waste disposal plan for special circumstances

5.0 Special Requirements

5.1 Equipment and Supplies Required

- 5.1.1 Biohazard waste red bag liners
- 5.1.2 Biohazard boxes (cardboard)
- 5.1.3 Yellow “incinerate only” or “pathological waste” labels (Obtained through Facilities Management)
- 5.1.4 Sharps Containers (plastic)
- 5.1.5 Red/Orange autoclave bags
- 5.1.6 Packing tape
- 5.1.7 Nitrile Gloves

5.2 Safety Requirements

- 5.2.1 Specified in the Laboratory-Specific Standard Operating Procedure (“SOPs”) and the BU Biosafety Manual

5.3 Training

- 5.3.1 Laboratory staff will receive annual Biological Waste training provided by the EHS (may be incorporated into Laboratory Safety Training).
- 5.3.2 Anyone who signs shipping papers will receive annual biosafety and triennial DOT shipment of biological waste training provided by EHS

5.4 Personnel Protective Equipment (“PPE”)

- 5.4.1 Specified in the BU Biosafety Manual for BSL1 and BSL2 agents and EHS PPE Selection Guide.

5.5 Medical Surveillance

- 5.5.1 Specified in the BU Biosafety Manual for the agents handled.

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
	Last Reviewed/Update Date: November 2015
Page #: Page 6 of 14	Approval Authority: IBC
Expiration Date:	
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

5.6 Other Prerequisites

5.6.1 Laboratory Safety Training (includes biological waste) is required annually for all laboratory staff generating biological waste. This training includes Bloodborne Pathogens Training.

5.6.2 Biological Waste Shipping Training (“Department Of Transportation”) is required every three years for those who sign biological waste shipping papers.

6.0 Applicable Locations

6.1 Waste Generation

6.1.1 All Boston University Charles River Campus BSL1 and BSL2 laboratories.

6.2 Waste Storage

6.2.1 See *Appendix A* for a Listing of Boston University Charles River Campus Biological Waste Storage locations.

7.0 Biological Waste Storage Areas

7.1 Storage areas shall be in an uncarpeted room or area with impervious, cleanable, non-absorbent flooring. The space must be used exclusively for waste storage. Additionally:

7.1.1 The storage area must have prominent signage indicating the space is used for the storage of regulated medical or biological waste;

7.1.2 The space must be designed or equipped to prevent unauthorized access;

7.1.3 The accumulation area must be located to protect the waste from the elements and prevent access by vermin;

7.1.4 There must be sufficient space to allow for clear separation of regulated medical or biological waste from any other waste.

7.1.5 The space must be adequate to accommodate the volume of regulated medical or biological waste generated prior to removal of waste for either waste transport off-site or on-site treatment, and

7.1.6 The space must be maintained such that there is no putrescence or off-site odors, using refrigeration when necessary.

7.1.7 Sharps shall be segregated from other biological wastes

8.0 Compactors or Grinders: Shall not be used to process medical or biological waste until it has been rendered noninfectious and safe for disposal in accordance with 105 CMR 480.150.

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
	Last Reviewed/Update Date: November 2015
Page #: Page 7 of 14	Approval Authority: IBC
Expiration Date:	
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

9.0 Accumulation Time Limit: All medical or biological waste must be treated on-site or transported offsite for treatment at a minimum once per calendar year.

10.0 Solid Biological Waste Collection

10.1 General Laboratory Solid Biological Waste: Includes all non-liquid, non-sharp, non-animal, and non-pathological wastes which are contaminated with RG1 or RG2 agents from a BSL1 or BSL2 laboratory. Any solid waste contaminated with genetically-altered RG1 or RG2 microorganisms or which contains recombinant or synthetic nucleic acid molecules is considered biological. This description includes but is not limited to, Petri dishes, plastic pipettes, soiled gloves and bench chucks. Unsoiled, uncontaminated gloves, bench chucks, and other uncontaminated materials must not be disposed of as solid biological waste. Risk Group 3 (“RG3”) and Risk Group 4 (“RG4”) contaminated wastes are managed under the provisions of a separate policy.

10.1.1 Laboratory personnel obtain cardboard ‘burn boxes’ and plastic biohazard bags from EHS. Both boxes and bags must be labeled with the biohazard symbol and the word ‘biohazard’.

10.1.2 Laboratory personnel use tape to construct ½ of the box, and line the open end of the box with two biohazard bags.

10.1.3 Contaminated solid biological waste is placed into the box by laboratory staff as it is generated.

10.1.4 Full bags are taped or tied closed (each bag), and the top of the box closed and sealed with tape.

10.1.5 Laboratory staff write the building and room number on the box using a permanent marker (building letter codes are OK).

10.1.6 The box is staged for pickup by EHS who transports the closed box to a designated storage room.

10.1.7 Laboratory personnel must immediately fill out an on-line pickup request form to notify EHS that a box is ready for storage.

10.1.8 Improperly closed, overfull, or leaking boxes will not be picked up, and must be repackaged by laboratory personnel.

10.1.9 Full, closed boxes are stored in designated storage areas until they are shipped for proper final disposal via a third-party vendor.

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
Page #: Page 8 of 14	Last Reviewed/Update Date: November 2015
Expiration Date:	Approval Authority: IBC
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

10.2 Contaminated Animal Wastes – Animal carcasses, parts, tissues and bedding which are infected with an agent, or from transgenic animals, or otherwise fall under the definition in section 3.1 (4) of this document.

10.2.1 Collection by laboratory staff as described in 10.1.

10.2.2 In addition, laboratory staff must place a yellow ‘incinerate only’ sticker on full, closed boxes containing contaminated animal wastes.

10.2.3 Laboratory animals which do not meet the definition in 3.1 (4) of this document should still be collected as biological waste. In these cases, the yellow ‘incinerate only’ sticker is not necessary.

10.2.4 Pickup is as described in 10.1 above.

10.2.5 Animal carcasses which are disposed of at the Laboratory Animal Care Facility (“LACF”) are stored in double-lined red bags in a dedicated freezer until picked up for disposal.

10.3 Pathological Wastes – Biological wastes including human parts, tissues organs as described in section 3.1 (2) of this document.

10.3.1 Collection by laboratory staff as described in 10.1.

10.3.2 In addition, laboratory staff must place a yellow ‘incinerate only’ sticker on full, closed boxes containing pathological wastes.

10.3.3 Pickup is as described in 10.1 above

11.0 Liquid Biological Waste Management

11.1 All liquid biological wastes (are decontaminated and disposed of inside the laboratory. Any liquid containing genetically-altered micro-organisms or which contains recombinant or synthetic nucleic acid molecules is considered biological waste.

11.2 Liquid waste is collected by the laboratory in labeled waste flasks or beakers. If a vacuum system is used, a double-flask with a High Efficiency Particulate Arresting (“HEPA”) filter between the vacuum line and outlet is employed.

11.3 Active liquid waste flasks or containers must have secondary containment adequate to hold their entire contents.

11.4 Prior to disposal, waste must be disinfected using an Environmental Protection Agency (“EPA”) registered agent per the laboratory SOP and BU Biosafety Manual. If bleach is used, a final bleach solution of at least 10% must be made, with at least 20 minutes of contact time.

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
Page #: Page 9 of 14	Last Reviewed/Update Date: November 2015
Expiration Date:	Approval Authority: IBC
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

11.5 After complete disinfection, waste liquid is discharged into a laboratory sink drain.

11.6 All liquid biological wastes must be disinfected at least daily.

12.0 Mixed Wastes

12.1 Biological wastes which contain radioactive or chemical contamination must be disinfected in the laboratory.

12.2 Chemical disinfectant, such as bleach, must be added to completely destroy potential pathogens that exist in the waste. Care must be used to prevent potential chemical reactions between the chemical or radioactive waste substance and the chemical disinfectant.

12.3 Biological waste with chemical or radioactive contamination must NEVER be autoclaved unless authorized by EHS.

12.4 Once a biological waste with chemical or radioactive contamination is disinfected, it must be managed as a radioactive or chemical waste.

13.0 Sharps Waste Management

13.1 Sharps, as defined in section 3.1(5) of this document, are collected in rigid, plastic containers provided to laboratories by EHS.

13.2 Containers are orange or red/orange in color, shatter-proof, leak-proof and puncture-proof, and marked with the universal biohazard label and the word 'biohazard'.

13.3 Laboratory staff place used sharps, whether contaminated or not, into these containers. When containers become full they are closed and staged for pickup.

13.4 An on-line pickup request should be made to EHS to collect the full sharps container and replace it with an empty one.

13.5 Overfull or open sharps containers will not be picked up until the laboratory has corrected the overfilling or closed the container.

14.0 Autoclaving Waste

14.1 Registration: As a rule, autoclaves are not to be used to disinfect RG1 or RG2 wastes from BSL1 or BSL2 laboratories. However, if the IBC determines that an RG1 or RG2 organism used in a protocol should be autoclaved prior to being sent for disposal as biological waste, the proposed autoclave unit must be registered with OEHS. See *Appendix B* for a listing of autoclaves authorized for the sterilizing of BSL1 and BSL2 biological waste.

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
Page #: Page 10 of 14	Last Reviewed/Update Date: November 2015
Expiration Date:	Approval Authority: IBC
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

- 14.2** Standard Operating Procedure: A written SOP for the sterilization of waste (may include animals and sharps) must be developed by the laboratory supervisor responsible for the use of the autoclave. The SOP must be in accordance with the BU Biosafety Manual and submitted to EHS as a part of the approval process.
- 14.3** Records: For each load or cycle including cycle time, pressure and temperature must be recorded in the Record-Keeping Log book provided by EHS.
- 14.4** Quarterly Autoclave Validation: The autoclave(s) used for sterilizing BSL1 and BSL2 waste will be validated quarterly by Laboratory Staff using a biological indicator. The Laboratory –specific SOPs shall include validation procedures.
- 14.5** Annual Calibration: Laboratories shall arrange for annual calibration/maintenance of autoclaves used for decontamination of biological waste.
- 14.6** Autoclaved Waste Transfer - All autoclaved waste will be removed immediately from the autoclave at the completion of the run. According to the laboratory-specific SOP, autoclaved waste will be packaged, labeled and stored as described in section 10.1 of this document.
- 14.7** Animal Waste - Infected animal carcasses and tissues are to be autoclaved only if allowed by the approved SOP. Autoclaved animals are repackaged for incineration according to 10.2 of this document.

15.0 Records and Forms

15.1 Autoclave Decontamination Logs

- 15.1.1** Standardized forms available through EHS,
- 15.1.2** Kept in binders at the site of the autoclaves. Forms must be consecutively numbered inside the binders.
- 15.1.3** Completed and maintained by the users of the autoclave,
- 15.1.4** Must include record of annual calibration, and
- 15.1.5** Kept on-site for 3 years.

15.2 Off-site Disposal Logs

- 15.2.1** Standardized forms available through EHS
- 15.2.2** Managed by EHS, and kept in binders in the EHS office.

15.3 Shipping Papers

- 15.3.1** Required by the Department of Transportation to be included with every shipment of biological waste offsite.

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
Page #: Page 11 of 14	Last Reviewed/Update Date: November 2015
Expiration Date:	Approval Authority: IBC
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

15.3.2 Shipping papers are provided by the vendor of biological waste disposal services.

15.3.3 Signed by custodial leads at the time of shipment, and are given to EHS for retention for 3 years.

15.3.4 When wastes are transported by EHS from one building to a storage area, a shipping paper is generated and kept on file by EHS.

15.4 Medical Waste Tracking Documents

15.4.1 Provided by the biological waste service provider as a receipt of final disposal of biological waste.

15.4.2 EHS verifies destruction of each shipment by receipt of Medical Waste Tracking Documents within 30 days of shipment.

15.4.3 Shipments for which Medical Waste Tracking Documents are not received within 30 days are reported to the Department of Public Health, and are investigated and resolved by EHS.

15.5 IBC Membership List and Meeting Minutes

15.5.1 The IBC meets on a monthly basis.

15.5.2 Minutes of the IBC meeting are shared online for public access

15.5.3 At least annually, the IBC reviews this waste policy

15.5.4 A list of IBC members is also kept by IBC office.

15.6 Training Records

15.6.1 Training records are managed by EHS and kept electronically or in hard copy form.

16.0 SOP Revision History

Version	Section / Paragraph Changed	Changes Made	Effective Date
V.1.1	Throughout.	SOP placed in new SOP format. Specific responsibilities incorporated.	
V.2	Throughout	Incorporated specific guidance on waste collection techniques based on DPH seminar.	

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
Page #: Page 12 of 14	Last Reviewed/Update Date: November 2015
Expiration Date:	Approval Authority: IBC
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

V.3	Formatting throughout	Corrected Microsoft-related formatting issues. Verified IBC comments.	1/09
V.4	No significant changes	No significant changes	2/2010
V.5	No significant changes	No significant changes	2/2011
V.6	Appendix A	Added 590 Comm storage room	4/2012
V.7	No significant changes	No significant changes	12/2013
V.8	Clarification throughout	Minor edits to make content more clear.	10/2014
V.9	2.1, 3.1, 10 and 11	Strengthened language regarding recombinant and synthetic nucleic acid molecules.	11/2015

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
Page #: Page 13 of 14	Last Reviewed/Update Date: November 2015
Expiration Date:	Approval Authority: IBC
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

APPENDIX A

Listing of Boston University CRC Waste Storage locations

Location	Service Area	Account Code	Stericycle Pickup	Capacity (boxes)	Description
OEHS Garage	Campus		At least monthly	100	A room has been constructed inside the garage for the storage of biological waste boxes.
LACF Freezer	LACF		At least monthly	5	Animal wastes from LACF are stored in this freezer.
590 Comm Ave Basement	590 Comm Ave		At least monthly	25	Dedicated waste storage room in an EHS-controlled section of the basement.

Unit: Environmental Health and Safety	SOP #: EM 101
	Revision #: 4
	Current Version Implementation Date: April 2012
Page #: Page 14 of 14	Last Reviewed/Update Date: November 2015
Expiration Date:	Approval Authority: IBC
SOP Title: BSL1 and BSL2 Biological Waste Management (CRC)	

APPENDIX B

Listing of Boston University CRC autoclaves authorized for the sterilizing of BSL1 and BSL2 waste.

Location	Laboratories Serviced	EHS Registration Number	Responsible Laboratory Supervisor