


Standard Operating Procedure			
	Ragon Institute BSL2+ Tissue Culture Waste Disposal Guidelines		SOP# LS-SOP-008
	Originated by:	Julia Bals, Thalia Bracamonte-Moreno	Date: 11/07/2019
	Reviewed by:	Vinay Mahajan, Grace Jia-Li Yuen, Alicja Trocha, Amruta Samant	Pages: 1 of 7
	Approved by:	Daniel Lingwood Shiv Pillai, Jessica Healey (EH&E)	

I) Purpose:

This standard operating procedure (SOP) describes standard waste disposal procedures in the BSL-2+ tissue culture (TC) rooms at the Ragon Institute (RI).

II) Scope:

This SOP applies to all BSL-2+ TC rooms at the Ragon Institute.

III) Responsibilities:


- A) Principal Investigators (PIs) are responsible for the overall implementation of this procedure in their laboratories.
- B) TC Leaders and Lab Managers are responsible for assisting in the implementation of this procedure and ensuring that the SOP is being followed in their research space.
- C) The onsite EH&E team is responsible for inspecting the TC rooms weekly, documenting and informing the TC Leaders, Lab Managers and/or PIs of any issues, and assisting with implementation of the SOP as needed.
- D) All members must follow this SOP and report any deviations immediately to their TC Leader, Lab Manager, or PI.

IV) Safety:

This document has been reviewed by RI BSL-2+ Lab Managers, EH&E Team, and PIBC members, and conforms to the BSL-2+ laboratory space guidelines.

V) Guidelines:


- A) **Approved Disinfectants**

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- a) Appropriate chemical disinfectants are D125 (1:64 dilution) **or** 10% bleach.
 - i) These concentrations are based on the total FINAL volume in the container, not the initial volume added to the container.
 - ii) **Only one disinfectant (D-125 or Bleach) can be used in each TC Room.**
 - iii) **MIXING D-125 and BLEACH PRODUCES TOXIC CHLORINE GAS!**
- b) After using 10% bleach to decontaminate the hood or other metal work surfaces, follow by wiping down surfaces with 70% ethanol to prevent metal corrosion.
- c) Bleach and D-125 solutions must be prepared daily to ensure maximum effectiveness.
- d) A 30-minute minimum contact time is required for freshly prepared bleach. A 10-minute minimum contact time is required for freshly prepared D-125.

B) Dry waste treatment

- a) Autoclave all dry waste before removing it from the TC room.
- b) Collect dry/semi-dry waste such as serologicals and pipette tips in buckets.
 - i) Buckets must be lined with a small autoclave bag.
 - ii) Do not add any disinfectant to the bucket.
 - (1) Autoclaving bleach produces chlorine gas. Do NOT autoclave anything that may contain bleach.
 - (2) Autoclaving D-125 produces irritating fumes. Do NOT autoclave D-125.
 - iii) Fill buckets to the marked fill line, or about $\frac{3}{4}$ full. Do NOT overfill buckets.
 - iv) Once the bucket is full, loosely tie autoclave bag over contents.
 - (1) Do not tightly close the bag or steam will not have a point of entry to adequately inactivate materials.
 - (2) Do not remove bag from bucket prior to autoclaving.
 - v) See section 4 for instructions on autoclaving dry waste.
- c) Large autoclave bags outside the hood are to be used for all other solid, non-sharp, biohazardous waste. When full, loosely tie the bag and place in the accumulation area.

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- i) Do not tightly close the bag or steam will not have a point of entry to adequately inactivate materials.
- ii) When disposing of non-sealable items that previously contained biohazardous waste (e.g., plates, petri dishes), aspirate any residual liquid prior to disposal in dry waste containers.

C) Liquid Waste


a) The following are acceptable liquid waste streams:

i) Vacuum flask

- (1) Fill with enough disinfectant to equal 1:10 dilution of bleach or 1:64 dilution of D-125 (e.g. 100 ml bleach or 16 ml D-125 per 1L waste).
- (2) Aspirate all liquids into vacuum flask.
- (3) Once flask is full, allow at least 30 minutes of contact time.
- (4) Thereafter, drain the contents of the flask into the sink with the water running.
 - (a) Lab coat, gloves, and eye protection are required when disposing of liquid waste.

ii) Liquids bucket


- (1) Fill with enough disinfectant to equal 1:10 dilution of bleach or 1:64 dilution of D-125.
- (2) Label the bucket with the date, the volume of disinfectant added, and your initials.
- (3) When the bucket is full, wait at least 30 minutes to achieve the minimum contact time.
- (4) Thereafter, drain the contents of the bucket into the sink with the water running.
 - (a) Lab coat, gloves, and eye protection are required when disposing of liquid waste.

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b) Do NOT autoclave liquid waste!

D) Autoclaving dry waste

- a) Wear a disposable lab gown if you are in the TC room.
- b) If you are in the anteroom, wear a white lab coat or a disposable gown in a different color that is easily distinguishable from the TC room gowns.
- c) Wear safety glasses, and a pair of gloves when using the autoclave.
- d) Close the autoclave anteroom door.
- e) Before loading cycle, ensure that autoclave waste bags have a small opening (~1 inch) to allow steam to enter and properly disinfect all waste.
- f) Do not overstuff the autoclave.
- g) Shut the autoclave TC door tightly once the autoclave is loaded.
- h) The best way to assure that the cycle will go through is to make sure that generator pressure is between 50-60 psi and jacket pressure is at 15 psi before selecting cycle (located on the front face of the autoclave /anteroom side)
- i) Run cycle (either gravity or liquid cycle). Cycle time should be set for a minimum of 30 minutes at 121C and 15psi.
- j) After the cycle is complete, slightly loosen the autoclave anteroom door to let steam escape. Keep your face away from the autoclave opening. Let contents cool for at least 30 minutes.
- k) Wear heat-resistant gloves when removing materials from the autoclave or handling contents.
- l) Dispose of autoclaved materials in a gray biohazard bin lined with a red bag.
- m) Transfer gray bins to final biohazard waste collection area for disposal.
- n) Wash hands or treat with Cal-stat before leaving the laboratory.
- o) If the autoclave in a TC room is not working, dispose of biohazardous waste using the following procedures:
 - i) Large dry waste items:
 - (1) Transport closed autoclave bags to another TC room for autoclaving as per TC-SOP-017.

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ii) Serological pipettes:

- (1) Remove serologicals from bucket and place in pipette coffin.
- (2) Move pipette coffin to gray biohazard bin and autoclave in another TC room. Follow TC-SOP-017 guidelines for proper transfer of BSL-2+ waste between TC rooms.

OR

- (1) Fill a clean liquid waste bucket with selected disinfectant (i.e., bleach or D-125).
- (2) Remove serologicals from the dry waste bucket and submerge in the disinfectant.
- (3) Let sit for at least 30 minutes.
- (4) Drain contents over the anteroom sink and dispose of serologicals in gray biohazard bins.


iii) All other small dry waste items:

- (1) Add selected disinfectant (i.e., bleach or D-125) to the dry waste bucket, completely covering the waste.
- (2) Allow the waste and disinfectant to sit for at least 30 minutes.
- (3) Remove the bag from the bucket and strain the contents over the sink with the water running.
- (4) Dispose of the bag in the gray biohazard bins.
- (5) Thoroughly rinse and dry the bucket to remove residual bleach.

p) Other autoclave precautions:

i) General Precautions:


- (1) Do NOT overload autoclave.
- (2) Do not allow autoclave bags to tightly touch the inside walls of the autoclave. They will melt.
- (3) Visually check the gasket for tears before each run.

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- (4) The drain valve must be emptied after every run. Any filtered contents on the mesh should be emptied. Carefully scrape and tap the inside.
- (5) If you must remove something from deep inside the autoclave, pull the metal rack out. Do not lean into the autoclave.
- ii) **DO NOT AUTOCLAVE ANY OF THE FOLLOWING:**
 - (1) Flammable, reactive, corrosive, toxic, or radioactive materials (including D-125, BLEACH, phenol, chloroform, trizol, b-mercaptoethanol, ethanol)
 - (2) Any liquid in a tightly sealed container.
 - (3) Paraffin-embedded tissue.
 - (4) Plastics such as polystyrene (PS), polyethylene (PE), and high-density polyethylene (HDPE).
- iii) When using kits or chemical reagents, check the MSDS for each reagent to ensure that they are suitable for autoclaving.

VI) Appendices/Notes:

- A) DO NOT AUTOCLAVE BLEACH. This includes anything that may still contain bleach residue.
 - a) This produces chlorine gas. This is a catastrophic, potentially life-threatening situation.
 - b) It also generates corrosive vapors that damage the autoclave.
- B) Do NOT autoclave D-125. The fumes are irritating and harmful.
- C) Ensure that your reagents do not react negatively with bleach and do not release any toxic byproducts.
- D) Do NOT mix bleach and D-125. Mixing bleach and ammonia compounds generates chlorine gas.

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I, _____, have read the above SOP and agree to adhere to the
 (PRINT NAME)

guidelines detailed therein. I understand that failure to adhere to these guidelines may result in revocation of TC access and repetition of the relevant biosafety training.

 (SIGNATURE)

 (DATE)