


<b>STANDARD OPERATING PROCEDURE</b>			
	<b>General Rules and Safety requirements in the BSL2+ laboratory areas</b>		<b>SOP#</b> LS-SOP-003
	Originated by:	EH&S	Date: 08 January 2019
	Reviewed By:	Amruta Samant	Pages: 1 of 3
	Approved by:	Amruta Samant	

**I. PURPOSE:**

The purpose of this procedure is to establish and outline the general rules and safety requirements in all BSL2+ laboratory areas at Ragon Institute.

**II. SCOPE:**

This procedure applies to all employees, contractors and trainees that are required to enter the laboratory facility.

**III. RESPONSIBILITIES:**


- A. The lab managers are responsible for the overall implementation of this procedure. The management needs to periodically reviewing the outlined procedure and initiated any updates to this procedure.
- B. All employees, contractors and trainees are required to follow this procedure.

**IV. SAFETY:**


This document outlines the guidelines to be followed in BSL2+ laboratory and some areas of BSL 1 laboratory.

**V. GUIDELINES:**

1. A disposable gown **MUST** be worn inside tissue culture (TC) rooms while working and in case of anyone else performing work in the area. If no work is being carried out in the TC lab, safety glasses and gloves must be worn. Gowns should be initialed and dated and needs to be discarded in biohazard containers every 7 days. They can be disposed off earlier in case of tear or spill.
2. A pair of gloves must be worn while entering the TC space and a second pair of gloves must be worn over the first in case of working in the biosafety cabinet. This second pair **MUST** be removed **EVERY SINGLE** time when exiting the cabinet.
3. Gloves should be regularly checked by visual inspection for cuts or tears and must be replaced accordingly.
4. It is **MANDATORY** to wear safety glasses when entering and working in the TC areas.
5. Before initiating any work in the biosafety cabinet (BSC), all areas should be wiped thoroughly with 70% ethanol and diligently including all equipment and tools inside the BSC. A bottle of freshly made D125 should be placed in the BSC. D125 must be used in case of any accidental spills. The area must be covered with D125 and gauze and left undisturbed for a period of 15 minutes after which the area must be wiped with 70% ethanol.
6. Hands must be washed prior to initialing any work at the end of work. Cal Stat may be used instead of hand washing.

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7. All door surfaces at Ragon Institute have the “No glove” policy. No common surfaces should ever be touched with gloves (whether new or contaminated).
8. Keeping the area neat, clean and tidy always helps and is recommended.
9. It is the responsibility of every user to make sure that the small bucket and vacuum flask are not full and over 7 days old. D125 **MUST** be changed every 7 days and a tape must be placed on the container with the initials of the person who has freshly prepared the D125 and the date of preparation. The big bucket under the cabinet **MUST** be changed as well. The directions for dilution of D125 are placed under each sink.
10. HEPA filters need to be **MANDATORILY** changed every 3 months.
11. No sharp items are permitted inside the tissue culture room. Should a need arise for any special experiment, an exclusive SOP must be drafted and then approved by lab management before use.
12. While working inside the laminar flow hood, rapid movements must be avoided so that air flow is not disrupted. It is recommended to adjust the chair height to its maximum level so that the user does not breathe in the air from the hood.
13. HIV seropositive and seronegative blood **MUST** always be spun in safety centrifuge canisters with the covers. The maximum volume in a 50.0 ml tube is 45.0 ml as leakages can easily occur in case the tube is overfilled.
14. After centrifugation, the inside of the containers and the centrifuge must be inspected for any potentials spills. In case of a spill, the entire bucket must be transferred inside the BSC and left undisturbed with D125 for a period of 15 minutes. All the liquid contents must be discarded in the small bucket with D125 and the container finally rinsed with 70% ethanol. This bucket should then be allowed to air dry before use.
15. Buckets inside the centrifuge must always have covers on during spinning and the conical and falcon tubes must have their tops on. It is **PROHIBITED** to spin glass tubes with blood, in which case the contents must be transferred to plastic tubes prior to initiation of any centrifugation.
16. When using strata coolers for freezing down cells, the coolers should be pre-cooled to 4<sup>0</sup>C overnight and they must never be used the same day of emptying their contents. Strata coolers should have a tag listed when they can be used by the next user.
17. Strata coolers must never be left at room temperature as this results in breakage of the coolers resulting in coolant leakage. They should be left in refrigerators for allowing them to cool.
18. CO2 incubators must be cleaned and the shelving must be autoclaved every 6 months.
19. It is **MANDATORY** to deep clean BSC every 6 month and the cabinet should have a tag indicating the name of the personnel who deep cleaned the hood and the date of cleaning.
20. Use of sandals or open toes shoes is **PROHIBITED** in the tissue culture laboratory. Usage of cellphones is **PROHIBITED** in the tissue culture room and in the anteroom. If you receive a call, you must exit the room (TC and anteroom) to answer it. A wall mounted phone is available in each TC room to use if necessary.
21. Use of headphones (one or two earbuds) is also **PROHIBITED** in the tissue culture laboratory.

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**GENERAL PRINCIPALS--How to a use Laminar Flow Safety Cabinet.**

Air is drawn into the cabinet and passed through efficiency Particle Air HEPA Filters. These filters remove all contaminants from the air, which sterilizes the air. The air is then used to create an airflow shield. This is the main barrier and protection. These cabinets are certified every 12 months to determine that airflow is at the proper velocity to ensure maintenance of the airflow barrier.

**VI. APPENDICES/ NOTES:**

General Precautions:

- Do not block the vents in the cabinet. These are the areas where air circulation occurs and this circulation is essential to your protection.
- Don't make any quick or sudden movements into or out the cabinet. You will create a countercurrent of airflow and will break the "curtain of air".
- Do not walk quickly behind someone working in a safety cabinet. These quick movements also create currents that disrupt the airflow barrier.
- Work as far inside the cabinet as you can. To protect your work and yourself, make sure you are doing all your work (opening, closing, manipulation with pipettes, etc.) at least 3-4 inches beyond the front vent.