STANDARD OPERATING PROCEDURE				
Ragon Institute of MGH, MIT and Harvard	**General Rules and Safety requirements in the Biosafety Level 2 (BSL2) laboratory areas**		**SOP#**	LS-SOP-005
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I. PURPOSE:

The purpose of this procedure is to establish and outline the general rules and safety requirements in all Biosafety Level 2 (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 review the outlined procedure and initiate 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 laboratories at Ragon Institute.

V. GUIDELINES:

1. Appropriate PPE should be worn when performing experiments in the BSL2 laboratory. This includes a lab coat and one pair of gloves when conducting work inside and outside of the biosafety BSC with non-infectious agents. Lab coats should be laundered as necessary. When working with infectious biological agents a dedicated lab coat or disposable gown should be worn. A new gown should be initialed and dated and needs to be discarded in biohazard containers every 7 days, if they become contaminated or lose integrity (tears, rips, etc). Gloves should be regularly checked by visual inspection for cuts or tears and must be replaced accordingly. Eye protection should be worn when transferring or working with infectious material outside of the BSC.
2. A dedicated BSC is to be used for all routine cell culture work (e.g. splitting cells); work with infectious agents or material within this BSC is not permitted. A dedicated 37C CO2 incubator is to be used for all non-infectious material; storage of infected or infectious material within this incubator is not permitted.
3. Before working in the BSC, areas should be wiped thoroughly with 70% ethanol, including all equipment and tools inside the BSC. When working with infectious material either 10% bleach solution or D125 should be placed within the BSC. The disinfectant must be used in case of any accidental spills. The area must be covered with disinfectant and gauze and left undisturbed for a period of 15 minutes after which the area must be wiped with 70% ethanol.
4. Hands must be washed or treated with Cal-stat after working with potentially infectious materials and before leaving the laboratory.

5. All door surfaces at Ragon Institute have the "No glove" policy. No common surfaces should ever be touched with gloves (whether new or contaminated).
6. Laboratories should be kept clean and free of clutter in accordance with standard biosafety practices.
7. 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. If the disinfectant being used is bleach, at least a 10% solution should be used. If the disinfectant being used is D125, it 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.
8. HEPA filters on vacuum flasks if used, must be changed every 3 months to ensure functionality.
9. Use of sharps should be minimized when working with infectious agents. Should sharps be required needles are never to be recapped and must be disposed of in a sharps container.
10. All potentially infectious BSL2 materials must be disposed of in gray Stericycle bins for treatment by laboratory waste vendor.
11. After centrifugation, the inside of the containers and the centrifuge must be inspected for any potential spills. When working with infectious agents or material, centrifuge buckets must be opened within a BSC. In case of a spill, the entire bucket must be transferred inside the BSC and left undisturbed with the disinfectant for a period of 15 minutes. All the liquid contents must be discarded in the small bucket with the disinfectant and the container finally rinsed with 70% ethanol. This bucket should then be allowed to air dry before use.
12. When working with infectious agents or materials, centrifuge buckets must always have aerosol-proof covers on during spinning and the conical and falcon tubes must have their tops on.
13. Freezing cells: When using a Mr. Frosty for freezing cells, isopropyl alcohol should be replaced every 3 freeze-thaw cycles. The container should be at room temperature prior to use. When using strata coolers for freezing down cells, the coolers should be pre-cooled to 4^0C 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. 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 and kept upside down.
14. CO2 incubators must be cleaned, and the shelving must be autoclaved every 6 months.
15. BSCs must be deep cleaned every 6 months and the cabinet should have a tag indicating the name of the personnel who deep cleaned the hood and the date of cleaning.
16. 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.
17. Use of headphones (one or two earbuds) is also PROHIBITED in the tissue culture and the anteroom spaces.

GENERAL PRINCIPALS--How to a use Biosafety 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".
- Keep your chair at an appropriate level so arms are perpendicular to the working surface and you have a clear view into the cabinet through the glass sash (you should not be "reaching" up or down)
- 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.