


STANDARD OPERATING PROCEDURE				
	<b>General Rules and Safety requirements in the Biosafety Level 2 (BSL2) laboratory areas when working with dengue virus serotypes 1-4</b>		<b>SOP#</b> LS-SOP-006	
	Originated by:	Aaron Schmidt	Date:	08 January 2018
	Reviewed By:	EH&E	Pages:	1 of 4
	Approved by:	Amruta Samant		

**I. PURPOSE:**

The purpose of this procedure is to establish and outline the general rules and safety requirements in when working with dengue virus serotypes 1-4 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 when working with dengue virus serotypes 1-4.


**V. GUIDELINES:**

1. When an individual is working with any dengue virus serotype they must observe the following BSL2 guidelines. Before initiating any work, the individual must put a sign on the entry to the tissue culture room alerting anyone who enters that dengue virus work is in progress and proceed with caution. Visitors from outside the Ragon Institute who are not trained to work in BSL2 conditions are not permitted to enter while virus work is in progress. Other non-infectious work is permitted to occur however appropriate PPE must be worn.
2. Disposable gowns cannot be reused and must be disposed of in biohazardous waste streams at the completion of work. If a tear or spill has occurred, replace immediately PPE immediately. Sleeve protectors or extra-long gloves can be worn at the discretion of the researcher (i.e. if your disposable gown does not fully cover your arms).
3. Gloves should be regularly checked by visual inspection for cuts or tears and must be replaced accordingly.
4. Safety glasses must be worn when manipulating the virus outside the BSC.
5. Before initiating any work in the BSC, all areas should be wiped thoroughly with 70% ethanol, including all equipment and tools inside the BSC. A 10% bleach solution should be placed in the BSC. The disinfectant must be used in case of any accidental spills. The area

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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.

6. Filter tips must be used for all experiments in case of aerosolization.
7. Filter tips should be discarded into a container containing 10% bleach solution (e.g. a recycled 500mL media bottle).
8. Serological pipets and aspirating pipets must be first decontaminated in a 10% bleach solutions before being disposed of in a separate biohazard bin.
9. The vacuum flask must always contain enough bleach so that the final bleach solution at any time is equal to 10%.
10. Once virus work is completed:
  - a. The vacuum flask must be emptied, and a fresh solution of bleach must be added.
  - b. All pipets, tube racks, etc within the BSC should be decontaminated with bleach, then 70% ethanol and placed in the center of the BSC.
  - c. The UV lamp should be turned on and signage saying that dengue-virus work has concluded with the time to allow at least 20 minutes for UV inactivation before new work in the BSC can occur.
  - d. All waste must be autoclaved at the end of the working day.
11. HEPA filters on vacuum flasks if used, must be changed every 3 months (or if there is a noticeable contamination with media) to ensure functionality.
12. For any use of sharps in the BSL2, an SOP must be drafted and then approved by lab management before use.
13. Buckets inside the centrifuge must always use aerosol-proof containers and loaded/unloaded in the BSC.
14. After centrifugation, the inside of the containers and the centrifuge must be inspected for any potentials spills. 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.
15. Hands must be washed or treated with Cal-stat after working with potentially hazardous materials and before leaving the laboratory.
16. All door surfaces at Ragon Institute have the “No glove” policy. No common surfaces should ever be touched with gloves (whether new or contaminated).
17. Laboratories should be kept clean and free of clutter in accordance with standard biosafety practices.
18. 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.
19. Use of headphones (one or two earbuds) is also **PROHIBITED** in the tissue culture and anteroom spaces.

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**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:**

- Transfer of dengue virus outside of the BSL2 laboratory must always be done using secondary containers that are aerosol-proof and clearly marked with biohazard stickers.
- All flavivirus stocks must be documented in the Virus Log Book that is kept in the TC room. This must include:
  - a. the name of the individual who prepared it
  - b. the virus serotype
  - c. cell-line origin
  - d. date
  - e. titre (in p.f.u./ml or f.f.u./ml)
  - f. number of individual vials (e.g. 0.5mL, 1mL) produced.
  - g. when an aliquot is removed the Virus Log Book must be updated with the initials of who removed it, the date and the remaining vials.
- Short-term storage of virus samples at 4C or -20C is permitted but should be kept in secondary containers, clearly marked of their contents and dated.

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 your 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.

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