

| Animal Care Services Standard Operating Procedure |   |                      |
|---|---|----------------------|
| <b>Document No:</b><br>14.11                      | <b>Subject:</b><br>Working Safely with Level 2 Biohazards |                      |
| <b>Date Issued:</b><br>January 2007               | <b>Revision:</b><br>3                                     | <b>Page No:</b><br>1 |

**Location:** Queen's University

**Responsibility:** Animal Care Services Staff

**Purpose:** The purpose of this Standard Operating Procedure (SOP) is to describe the procedure for the care of rodents housed in a Level 2 biohazard room (B265, B265B, B265C).

## 1. Introduction:

- a) There are four different containment levels as defined in the Canadian Biosafety Standard (CBS) by Public Health Agency of Canada. The level of containment required is determined by the Risk Group of the particular agent and outlines the procedures that are used while working with the agent.
- b) Approval is based upon information provided in your Biohazard Permit and is used only as stipulated in the Animal Use Protocol (AUP).
- c) The primary containment devices for CL2 work within ACS include certified biological safety cabinets (BSC), appropriate personal protective equipment (PPE), hand washing, CL2 waste collection, disposal and decontamination protocols.
- d) Eating, drinking, storing human food or utensils, applying cosmetics and inserting or removing contact lenses is not permitted. No oral pipetting is allowed.
- e) All procedures are conducted in the biological safety cabinet (BSC) that has been certified within the last year. This includes injections, anesthesia, euthanasia and all cage changes.
- f) 0.5% Accelerated Hydrogen Peroxide (AHP) is used as the chemical for decontamination.
- g) When you enter the room, turn on the BSC and let it sit for a minimum of 5 minutes, check to ensure it is not alarming prior to use. If alarming, do not use and notify Associate Director, Animal Care Services. See EH&S SOP-Biosafety-03-Biological Safety Cabinets.

**Abbreviations:** Animal Care Services **ACS**, Principal Investigator **PI**, Biological Safety Cabinet **BSC**, Accelerated Hydrogen Peroxide **AHP**

---

| Animal Care Services Standard Operating Procedure |   |                      |
|---|---|----------------------|
| <b>Document No:</b><br>14.11                      | <b>Subject:</b><br>Working Safely with Level 2 Biohazards |                      |
| <b>Date Issued:</b><br>January 2007               | <b>Revision:</b><br>3                                     | <b>Page No:</b><br>2 |

## 2. Room Entry and Exit Procedures:

- a) Entry is restricted to trained animal care staff and research personnel.
- b) PPE requirements:
  - i. Shoe covers
  - ii. disposable gown
  - iii. hair bonnet
  - iv. double gloves
  - v. level 2 ASTM surgical mask (if handling LCMV infected animals or contaminated bedding, fit tested N95 mask is required)
- c) Entry: PPE is donned in the anteroom before entering the room. The BSC is checked to ensure it is on and working properly before use.
- d) Exit: PPE is removed and discarded into the autoclave bin at door and hands are thoroughly washed at the sink in the anteroom.
- e) PPE is removed in the following order:
  - i. Shoe covers
  - ii. 1<sup>st</sup> pair of gloves
  - iii. Gown
  - iv. Hair bonnet
  - v. Next pair of gloves
  - vi. Mask

## 3. Rodent Care:

- a) Cages are housed on a ventilated rack, with inward directional air flow.
  - b) Mice are checked daily for signs of illness, any concerns are reported to the lab staff and a clinical case report is generated.
  - c) Animal health checks are performed by visualizing the mice through the cage. If the cage is to be opened, it is brought to the BSC where the lid can be removed. When finished the cage lid is closed while in the BSC, and then the cage is placed back on the rack in its designated space.
  - d) Any deaths are reported on the room mortality log and to the investigator.
  - e) Carcasses must be removed from the cage and are double bagged, labeled with a biohazard sticker, and left in the BSC for the laboratory personnel to pick up (if needed), otherwise they are placed into the carcass freezer.
  - f) The cage must sit properly on the rack to be fully engaged for appropriate airflow and access to water.
-

| Animal Care Services Standard Operating Procedure |   |                      |
|---|---|----------------------|
| <b>Document No:</b><br>14.11                      | <b>Subject:</b><br>Working Safely with Level 2 Biohazards |                      |
| <b>Date Issued:</b><br>January 2007               | <b>Revision:</b><br>3                                     | <b>Page No:</b><br>3 |

#### 4. Supplies:

- a) Supplies in the holding room must be kept to a minimum.
- b) Once inside the room, supplies must be decontaminated if they are to be removed (i.e. wiped with peroxide and allowed appropriate contact time or prepared for autoclaving by ACS) before bringing them out of the room.
- c) All dirty cages must have the lids securely on the cage, the outside of the box is to be sprayed with 0.5% activated hydrogen peroxide and left in the designated area of the holding room for ACS staff to process.

#### 5. Cage Changing:

- a) Ensure gloved hands are thoroughly cleaned with 0.5% activated hydrogen peroxide in between cages, before and after handling caging and mice.
  - b) Cages are changed once every second week. Water bottles (if used) are autoclaved and changed once a week.
  - c) The cage is opened by removing the lid and placing it top side down beside the cage.
  - d) The food and enrichment devices and mice are transferred to the clean cage.
  - e) The cage lid is put back on the cage.
  - f) The clean cage is then placed back on the rack in its designated space.
  - g) Stack the dirty boxes in the hood until you have changed four boxes. Make sure to place a chemical indicator strip in the middle of your stacked boxes. Working in the hood, place a filter top lid on the stack of boxes (found in room), place boxes in an autoclavable clear bags, tie and place biohazard labels for autoclaving. Remove the bag/boxes and spray the outside with 0.5% activated hydrogen peroxide and place in the designated area for dirty caging.
  - h) Continue working this way until all boxes are changed and dirty boxes are bagged and ready for autoclaving. Leave boxes in the room overnight if it's late in the day and you are unable to autoclave immediately.
  - i) Bags are sprayed with 0.5% activated hydrogen peroxide before removal from the room, place bags on autoclavable cart, take the cart to the autoclave, don mask (N95), and gloves to untie bags (to ensure steam penetration) and autoclave immediately.
  - j) As required for validation, place a biological indicator inside one of the cages (use a stack of clean cages to allow easy retrieval without risking contamination
-

| Animal Care Services Standard Operating Procedure |   |                      |
|---|---|----------------------|
| <b>Document No:</b><br>14.11                      | <b>Subject:</b><br>Working Safely with Level 2 Biohazards |                      |
| <b>Date Issued:</b><br>January 2007               | <b>Revision:</b><br>3                                     | <b>Page No:</b><br>4 |

when removing it) as close to the middle of the autoclave as possible, retrieve once autoclaved, incubate 24hrs and record the results. Autoclave load remains in the autoclave room untouched until results are known. If the autoclave load was successful, cages can be taken to the cage washer room for processing. If autoclaving was unsuccessful repeat this procedure until successful.

- k) For autoclave safety manual see EH&S SOP-Biosafety-09-Autoclaves Biohazardous Waste Treatment, for operating instructions, see SOP #1.19: Maintenance and Operation of Autoclave.
- l) When water bottles are used, the water is collected and added to a bleach solution reaching a minimum final concentration of 10% bleach for decontamination prior to disposal. The empty bottles are autoclaved.

## 6. Room Maintenance:

- a) Automatic Watering:
    - i. Be sure to flush system for 2 minutes daily
    - ii. Check the pressure reducing station to ensure the PSI is between 3 and 7psi
    - iii. Replace leaking spigots and toggle new spigots to ensure water is available
  - b) Room gauges are checked daily for temperature and pressure, parameters are recorded on the room sheet.
  - c) Eyewash station checked weekly, letting it run for 3-5 minutes.
  - d) Emergency shower in room B264C checked and run for 3-5 minutes weekly to ensure proper functioning and to flush the drain.
  - e) Mop floors daily using 0.5%w/w ready to use Accelerated Hydrogen Peroxide using disposable mop heads. The disposable mop head acts as a sweeper. The moistened debris is collected into one corner of the room while mopping and is then picked up with the mop head and disposed of daily.
  - f) Wipe down all counters and clean sinks weekly.
  - g) Stock supplies weekly or as needed.
  - h) Hoods are cleaned weekly using 0.5% Accelerated Hydrogen Peroxide.
  - i) Vent rack blowers are checked daily for alarms and to ensure filters do not require changing.
  - j) Garbage is collected daily, double bagged and the outer bag is sprayed with peroxide prior to removal from the biohazard suite and placed into the grey biohazard bins in the B2 dirty side hallway. Excess peroxide must be mopped
-

| Animal Care Services Standard Operating Procedure |   |                      |
|---|---|----------------------|
| <b>Document No:</b><br>14.11                      | <b>Subject:</b><br>Working Safely with Level 2 Biohazards |                      |
| <b>Date Issued:</b><br>January 2007               | <b>Revision:</b><br>3                                     | <b>Page No:</b><br>5 |

up off the floors immediately after spraying bags down to avoid a chemical build up on the floors.

***SOP Revision History:***

| Date       | New Version                          |
|------------|--------------------------------------|
| 02/01/2021 | Triennial Review                     |
| 06/01/2022 | SOP Revision – removed Clinicide use |
| 02/26/2025 | UACC Review; Updated Template        |
|            |                                      |
|            |                                      |
|            |                                      |
|            |                                      |
|            |                                      |
|            |                                      |
|            |                                      |
|            |                                      |