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## 1.0 PURPOSE

The microbiology laboratory in Keenan Research Centre of Biomedical Science (#644) is designated for culturing **Risk Group 2 (RG-2)** bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and the culturing of large volumes, defined as **over 3L of any RG-1** bacteria. Culturing more than 10L of any RG-2 bacteria is not permitted at the LKSKI.

## 2.0 PROCEDURE

### User Guidelines

#### **Requirements for use of the Bacterial Culture Room**

- The principal Investigator (PI) must have an active Research Biosafety permit.
- All bacteria culture room users must have received training from the RCF Specialist responsible for the Microbiology Room. Training is required to obtain access to the room.
- If the labs plan to use RG-2 bacteria or more than 3L of RG-1 bacteria, the Standard Operating Procedure (SOP) should be approved by the research biosafety officer. Such users must post a notice on the BSC indicating the use of RG-2 pathogen. Additionally, they must sign up to use the BSC and incubators in the Microbiology Laboratory.

#### **Personal Protective Equipment**

- Gloves
- Lab coat with purple collar
- Mask (if appropriate)
- Eye protection (when working with large volumes of RG2) how much?

Wear proper personal protective equipment at all times within the room. Don the gloves once inside the room. Gloves should always be worn when handling samples, flasks, etc., in the BSC and incubators. Users must discard gloves into the appropriate garbage before exiting the microbiology room.

### ***Work Practices***

Follow the Canadian Biosafety Standards and Guidelines (CBSG) and the Biological Safety Cabinet Guidelines. The following practices are also required:

### ***BSC in the Microbiology Room***

- Work that is highly likely to generate bioaerosols must be performed in the Biological Safety Cabinet (BSC).
- Before and after using the BSC, disinfect the interior with 70% ethanol. Please note that sterilization using UV light is not recommended.
- If the BSC is non-functional, do not use it and report it immediately to the RCF Specialist.

### ***Bacteria Culture Safety Practices***

- Doors to the bacteria culture room must be closed at all times.
- If you are using RG-2 or a large volume of RG-1 bacteria, a notice must be posted on the door to room 644 warning other workers of the work being carried out inside.
- Label EVERYTHING. Include your initials, date, pathogen nomenclature, and any other information you need to add. For long-term experiments, update the date on your sample plates if you've made any changes, such as changing media.
- DO NOT leave unlabeled materials in the room. They will be thrown out if found!
- Wash your hands before going into the room and after removing your gloves.
- Perform procedures in a way that minimizes aerosols. Follow practices such as opening tubes slowly, pouring liquids without splashing, etc.
- Don't accumulate plates and tubes in the microbiology lab. Throw away old cultures once you have the bacteria you need.
- Report equipment problems and contamination outbreaks to the RCF specialist.

### ***Proper waste disposal and decontamination***

- Decontaminate all surfaces you have used after finishing your work, including the BSC and bench top, with 70% ethanol.
- Segregate solid biological waste from the microbiology room appropriately into a yellow biohazard bag or a yellow receptacle/sharps container.

- All liquid biological waste must be disinfected using 1:10 bleach in an appropriately sized container for 30 minutes before drain disposal. Please DO NOT use bleach on any work surface; it is solely for disinfection of liquid waste.

### ***Transport and storage***

- Use a secondary container for transport.
- Do not store cultures or plates with bacteria in the microbiology lab.

### ***Emergency procedure***

#### ***Small spill in lab***

- If the spill is 100 ml or less of RG-1 bacteria, cover the spill with paper towels. Discard paper towels in biohazardous waste. Clean and dry the area and further disinfect with 70% ethanol.

#### ***Large spill in lab- more than 100 ml of RG1 or any volume of RG2***

- Alert any other occupants and evacuate the room for 30 minutes until all aerosols have settled before cleaning the spill. Notify the RCF specialist and the research biosafety officer. Secure the area to avoid traffic.
- Don appropriate PPE (gloves, lab coat, goggles/face shield, mask) before entering the affected area.
- Remove any sharps using forceps or scoop and place them in a biohazard sharps container.
- Most disinfectants are less effective in the presence of high concentrations of protein, absorb the bulk of the spill by placing paper towels or absorbent material. Use forceps to discard paper towels or absorbent materials into a yellow biohazardous bag.
- Clean the spill site with 1:10 bleach, ensuring you reach all limits of the spill. Leave for 30 minutes and then absorb the liquid using paper towels or absorbent material. Repeat if necessary. Discard all waste into a yellow biohazard bag.
- Wipe the area until dry.
- Discard the mask and gloves in the yellow biohazard bag and loosely close the bag. Place the lab coat in an autoclavable bag and autoclave. If any other item of clothing is contaminated, remove it carefully, folding the contaminated area inwards. Goggles and other reusable items should be soaked in 70% ethanol for 30 minutes, rinsed, and air dried.
- Wash hands thoroughly. Notify your supervisor, RCF specialist and Biosafety Officer.

### **Spill in BSC**

- If a spill occurs in the BSC follow the BSC spill response procedure guidelines outlined in the *Biological Safety Cabinet Guidelines*.

### **First Aid**

- In case of accidental splash or inoculation/mucosal absorption, immediately wash the area with soap and running water for a minimum of 15 minutes.
- If eyes get potentially contaminated, immediately flush the eyes at an eyewash station for a minimum of 15 minutes.
- For accidental ingestion, please report to the Emergency Room.

### **DEFINITIONS**

Term/Acronym	Definition
<b>PPE</b>	Personal Protective Equipment
<b>BSC</b>	Biosafety cabinet
<b>RG</b>	Risk Group
<b>SOP</b>	Standard Operating Protocol

### **3.0 REFERENCES**

Government of Canada. (2015). Canadian Biosafety Standard (2nd ed.). Ottawa, ON, Canada: Government of Canada.  
<https://health.canada.ca/en/epathogen>

Version	Approval/Sub-approval body	Approval date
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