St. Michael's

Inspired Care. Inspiring Science.

Title:	**Bacterial Culture Guidelines**		
Program or Department:	Research	**Document Type:**	PROCEDURE
Effective Date:	January 01,2015	**Author**	Steven Hayes
Next Review Date:	January 01,2016	**Reviewing Body:**	Research Biosafety Committee
Emergency Code:		**Approving Body:**	Research Biosafety Committee
Keywords:	Bacteria,Bacterial , Culture , Biosafety , Laboratory	**Document Number:**	0000001048

Introduction

Room number 647 (Bacterial Culture room) at the LKSKI has been designated for the culturing **Risk Group 2 (RG-2)** bacteria such as *Pseudomonas aeroginosa* and for the culturing of large volumes, defined as **over 3L of any RG-1** bacteria. Please note that culturing in excess of 10L of any RG-2 bacteria is not permitted at the LKSKI. As with all the wet bench spaces within the LKSKI, room 647 is a **Containment level 2** (CL-2) area.

Associated Procedure

User Guidelines

Requirements for use of the Bacterial Culture Room

- Principal Investigator (PI) must have an active Research Biosafety Committee (RBC) permit
- PIs and researchers working with the cell lines must have up-to-date biosafety and WHMIS training
- All bacteria culture room users must have received training from RCF Coordinator responsible for the Bacterial Culture Room. Access to the room will only be provided after training. Contact the Research Facilities Secretary to activate your access card
- A copy of the RBP must be available at all times in the room. A copy of the Standard Operating Procedure (SOP) for bacteria culture must also be present in the room

Booking information for the Bacteria culture room

- All users have to sign up for the use of the BSC, incubator, shaker incubator and autoclave in the Bacterial Culture Laboratory

Personal Protective Equipment

1. Gloves
2. Lab coat with purple collar
3. Mask (if appropriate)

4. Eye protection (if appropriate)

Proper personal protective equipment must be worn **at all times** within the room. Only put on the gloves once inside the room. Gloves should always be worn when handling samples, flasks, etc. in the BSC and incubators. Gloves must be discarded into the appropriate garbage before exiting the tissue culture room.

Work Practices

Follow the Canadian Biosafety Standards and Guidelines (CBSG) and the Biological Safety Cabinet Guidelines SOP (where necessary) when working in the bacteria culture room. The following practices are also required:

BSC in the Tissue Culture Room

- Work that is highly like to generate bio-aerosols must be performed in the Biological Safety Cabinet (BSC). Before and after using the BSC disinfect the interior with a 10% Virox solution (Accelerated Hydrogen Peroxide). Please note that sterilization using UV light is not recommended. If the BSC is non-functional, do not use and report it immediately to the 6th floor RCF coordinator.

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, post a notice on the door to room 664 warning other workers of the work being carried out inside.
- Label **EVERTHING**. Include your initials, date, cell line nomenclature and any other information that 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 when you are not in the room. It will be thrown out if found!
- Wash your hands before going into the room and after removing your gloves
- Perform procedures in such a way that minimizes aerosols. Follow practices such as opening tubes slowly, pouring liquids without splashing etc.
- Don't accumulate plates and tubes. Throw away old cultures once you have the bacteria you need
- Report equipment problems and contamination outbreaks to the RCEF Coordinator so that appropriate actions can be taken

Proper waste disposal and decontamination

- Decontaminate all surfaces all surfaces you have used after finishing your work. This includes the BSC and bench top. Virox is supplied in the room
- All biological waste from the tissue culture room must be placed in a yellow biohazard bag or a yellow receptacle/sharps container for waste that may puncture. overfill bags or sharps containers as this can cause injury to others who may accidentally come into contact with them
- If you have liquid waste, disinfect by using 10% (v/v) Bleach, in an appropriately sized container for 30 mins. After this time the waste may be disposed of down the drain. **DO**

NOT use bleach on any work surface, it solely for disinfection of liquid waste

Transport and storage
Follow the SMH Policy for transporting infectious and hazardous materials.
Storage will vary depending on the bacteria and its use.

Emergency procedure

BSC

- If a spill occurs in the BSC follow the BSC spill response procedure guidelines outlined in the BSC Guidelines. Notify the RCF coordinator for the floor

Spill in the open lab:
If the spill is **100mls or less** of RG-1 bacteria, clean spill using paper towels soaked in concentrated Virox, then dispose of the paper towel in biohazardous waste. Notify the RCF coordinator.

If the spill is **greater than 100mls of RG-1 or any volume of RG-2 bacteria:**

- Alert any other occupants and evacuate the room for 30 minutes until all aerosols have settled before going back to clean the spill. Notify RCF coordinator and the Biological Safety Officer. Secure the area to avoid traffic
- Put on all appropriate PPE (gloves, lab coat, goggles/face shield, N95 respirator) before entering affected area
- Remove any sharps using forceps or scoop and place in a biohazard sharps container
- Most disinfectants are less effective in the presence of high concentrations of protein, so absorb the bulk of the spill by placing paper towels or absorbent material around the exterior on the leak, then you're your way inwards towards the center of the spill. Use forceps to discard paper towels or absorbent materials and place in a yellow biohazardous bag
- Clean the spill site with 10% Virox, ensuring you reach all limits of the spill. Leave for 30 minutes and then absorb the liquid using paper towels. Repeat if necessary. Discard all waste into a yellow biohazard bag
- Wipe the area until dry
- Discard N95 respirator 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 and carefully folding the contaminated area inwards. Goggles and other reusable items should be soaked in a 10% Bleach for 30 minutes, rinsed and allowed to air dry
- Wash hands thoroughly
- Complete an incident report via the online event tracker
- Notify your supervisor

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

Risk Group categorization

Risk Groups (RG) are defined as the inherent risks of a pathogen based on a variety of factors, including severity of disease caused, availability of effective treatments or vaccines, virulence and whether the pathogen is indigenous to Canada. A full description of the Risk Group classification system is given in the **Risk Group Classification SOP**.

Containment Level

Containment Level classification provides workers with the engineering, procedural, operational and technical requirements required for working with a specific pathogen. A full description of the Containment Level classification is given in the **Containment Level SOP**.

Revision Number	**Contact**