***STANDARD OPERATING PROCEDURE – B007***

**Working with Risk Group 2 microorganisms**

1. **Objectives**

The objective of this document is to establish standard operating procedures for working with Risk Group 2 microorganisms, ensuring the safety of laboratory personnel by mitigating potential risks associated with hazardous materials and injuries. Additionally, this SOP aims to enhance the efficiency of experimental workflows.

1. **Personal Protective Equipment**

To ensure safety when working with Risk Group 2 microorganisms, appropriate personal protective equipment (PPE) must be worn. This includes:

* Long pants and closed-toe shoes to protect against spills and splashes.
* A long-sleeved, buttoned lab coat to minimize skin exposure.
* Safety glasses or goggles to protect against splashes or flying debris.
* Disposable nitrile gloves to prevent direct contact with hazardous materials.
* If the user has long hair, it should be tied back.

1. **Potential Hazards**

Working with Risk Group 2 microorganisms presents various hazards that must be managed to maintain a safe working environment. This includes:

* **Biological Hazards:** Infection risk through skin contact, mucous membrane exposure, inhalation of aerosols, or accidental ingestion. **Allergic reactions** to microbial components or culture media. **Cross-contamination** between samples due to poor aseptic technique.
* **Physical Hazards:** **Burns** from autoclaves, Bunsen burners, or hot media.
* **Cuts/lacerations** from broken glassware.
* **Slip hazards** from liquid spills.
* **Chemical Hazards:** **Exposure to disinfectants** (e.g., ethanol, bleach) used in decontamination procedures.
* Workers with **pre-existing conditions,** including but not limited, e.g. allergies, immunocompromised states, chemical sensitivities, or those who are pregnant or planning pregnancies should notify their supervisors and medical specialists. Should any concerns be expressed by these workers, this task should be stopped immediately.

Laboratory workers with **pre-existing conditions,** including but not limited to allergies, immunocompromised states, chemical sensitivities, or those who are pregnant or planning pregnancies should notify their supervisors and medical specialists. Should any concerns be expressed by these workers, their job duties and activities should be reviewed.

1. **Training**

Ensure all personnel have received proper training on their hazards and safe handling techniques. Undergo medical surveillance and register as a biohazard worker prior to the start of work.

* MC06 Biological Safety
* MC03 Chemical Safety II / Hazardous Waste Management
* MC07 Chemical Safety I / Chemical Safety for Laboratory Users
1. **Procedures**
2. Preparation
* Work in a BSL-2 approved laboratory facility.
* Familiarize yourself with the locations of emergency equipment, emergency exits, and evacuation procedures.
* Read the pathogen safety data sheet (SDS), and / or scientific literature by acknowledging the risks associated with the organisms to be handled.
* Ensure prior training in microbiological methods, particularly aseptic techniques, or receive close supervision by someone with microbiological training.
* Work bench should be clear of clutter and personal possessions (e.g. no bags, clothing). Only the equipment necessary for the tasks to be performed should be present on the bench.
* Disinfect the bench surface and any tools required (e.g. the surface of Pipetman-type pipettes) with 70% to 80% (v/v) ethanol solution before the commencement of lab work.
1. During Culturing and Manipulation
* Never eat, drink, smoke, or apply cosmetics in the microbiological lab.
* Do not use mobile phones during lab work as they may act as a vector to carry microbes out of the lab.
* For bacterial manipulations, sterilize the microbiological loop immediately before each procedure by flaming in a Bunsen burner.
* Media and equipment should be sterilized by autoclaving prior to use.
* Sterilize the glass spreader immediately before each procedure by dipping in 100% ethanol and briefly flaming using the Bunsen burner.
* Turn off the Bunsen burner when not actively doing lab work. Do not leave a Bunsen flame unattended.
* Work close to the Bunsen flame when making transfers of cultures, streaking plates, etc. to minimize deposition of airborne microbes onto / into media.
* The biosafety cabinet is required for manipulations of Risk Group 2 organisms that are likely to generate aerosols including pipetting, sonicating, vortexing, centrifugation, and opening conical or microcentrifuge tubes.
* Label all cultures with organism details (including species name and strain), worker's name, and culture date. Incubate cultures in designated locations and avoid removing them from the lab.
1. Post-Culturing
* Upon finishing work, sterilize the bench again with 70% to 80% (v/v) ethanol, and discard all unwanted cultures into the appropriate location.
* All microbial cultures must be **autoclaved** prior to discarding and cannot be poured down the sink or disposed of as regular rubbish.
* Wash hands with antiseptic soap before leaving the lab.
* Remove the lab coat or gown before leaving the laboratory. For a BSL-2 laboratory, lab coats should not be taken out of the lab unless it is autoclaved first.
1. Transportation and Storage
* Label all cultures with organism details (including species and strain), worker's name, and culture date. Incubate cultures in designated locations and avoid removing them outside the lab.
* Transport Risk Group 2 cultures inside an unbreakable sturdy container (i.e. a strong plastic box with a lockable lid) if they need to be taken out of the BSL-2 lab.

**6) Spills or Incident Reporting**

* All biohazard spills must be cleaned up following Standard Operating Procedure 002 - Cleanup of Biohazard Spills.
* Any spills, accidents, or near misses must be reported to the PI and/or the departmental safety officer (DSO) immediately.
* In the case of serious incidents, immediately inform the Security Unit by calling the 24-hour hotline on **2358 8999.**

**7) References**

* Campbell, L. (2016). *SOP\_SMB026: Working with Risk Group 2 microorganisms.* Risk Assessment. The University of Sydney.
* Coleman, N. (2014). *SOP SMB026.2 (NC 0714): Working with Risk Group 2 microorganisms.* Standard Operating Procedure. The University of Sydney
* Safety and Environmental Protection Manual *- Chapter 9: Biological Safety | Health, Safety and Environment Office - the Hong Kong University of Science and Technology*