***STANDARD OPERATING PROCEDURE – B003***

**Use of Class II Biological Safety Cabinet**

1. **Objectives**

The objective of this document is to establish standard operating procedures for the use of class II biological safety cabinet (BSC), ensuring the safety of laboratory personnel by mitigating potential risks associated with hazardous materials, and injuries. In addition, this SOP aims to enhance the efficiency of experimental workflows.

1. **Personal Protective Equipment**

To ensure safety during the use of class II BSC, appropriate personal protective equipment (PPE) must be worn. This includes:

* Long pants and closed-toe shoes to protect against spills and contamination.
* A long-sleeved, buttoned lab coat to minimize skin exposure.
* Disposable nitrile or latex gloves to prevent direct contact with hazardous materialsIf the user has long hair, it should be tied back.
1. **Potential Hazards**

The use of class II BSC presents various hazards that must be managed to maintain a safe working environment. These include:

* **Infection Risk:** Incorrect use of the cabinet or improper handling of GMOs and infectious materials may lead to the release of pathogens into the environment.
* **Fire Hazard:** Use of ethanol for sterilization or improper use of Bunsen burners or other open flame sources can lead to burns or fire.
* **Explosion Risk:** Improper use of ethanol and leak of LPG may cause explosions.
* **UV Light Exposure:** Direct exposure to UV light can cause eye injury or skin burns.
* **Allergic Reactions:** Contact with infectious agents may trigger allergic reactions or pose risks to unborn children, including malformations or death.
1. **Procedures**
2. Preparation
* Check the certification date of the BSC, located at the front of the cabinet. If expired, do not use the cabinet and contact CMO/LS by ext. 6847 or send a defect report.
* Turn off the UV light if it is in use.
* Turn on the BSC fan at least 10 minutes before starting work to allow for proper air filtration. Ensure nothing obstructs the front grille. Adjust the sash to the recommended height.
* Ensure the drain valve is closed.
* Check the Magnehelic gauge to confirm HEPA filter operation. It should read within 0.2 of the value on the certification sticker. If not, do not use the cabinet and contact CMO/LS.
* If the cabinet has an alarm, test it and switch it to the “ON” position.
* Confirm inward airflow by holding a piece of tissue at the middle edge of the viewing panel. It should be drawn inward into the BSC. Ensure the area facing the BSC and BSC opening is clear of any obstructions.
* Turn on the light switch for the lamp to ensure sufficient lighting inside the cabinet.
* Clean the cabinet surface and decontaminate all materials with 70% ethanol before placing them inside the BSC.
* Position larger items (e.g., biohazard bag holder, pipette tip box) toward the back and sides of the cabinet. Place aerosol-generating equipment at the back.
* Wear appropriate PPE to protect yourself and your samples from contamination.
* Re-check the Magnehelic gauge and verify the front airflow at the grill by holding a disposable wiper such as Kimwipe as the flow indication.
* Adjust stool height so that your chin is level with the bottom of the sash.
* Allow the work zone air to purge for at least five minutes before starting work.
1. Working Inside the Class II Biological Safety Cabinet
* Keep doors to the room closed.
* Do not work in the BSC if a warning light is visible or an alarm is activated.
* Ensure that all activities progress from clean to contaminated areas across the work surface.
* Move arms slowly in and out of the cabinet, keeping them perpendicular to the front opening to avoid disrupting the air curtain and laminar flow.
* Avoid rapid arm movements or sweeping motions.
* Remind other researchers to not walk quickly in front of the cabinet while work is being conducted inside the BSC.
* Do not place items on the front grill or block the air openings at the back of the cabinet.
* Keep supplies, equipment, and papers well away from the BSC to ensure air intake and grills are unobstructed.
* Center your work towards the middle of the cabinet, ideally at the air split or at least six inches (approx. 15 cm) from the front grill.
* Avoid bringing non-essential equipment and supplies into the cabinet.
* Always use mechanical pipetting aids and never perform mouth pipetting.
* Avoid using heat sources like Bunsen burners or other open flames inside the BSC, as they can disrupt laminar airflow. Instead, use single-use bacteriological loops, or micro-burners for sterilizing re-usable bacteriological loops.
* When finished work in BSC, discard disinfected waste, including gloves, and utilize an in-line HEPA filter to protect the vacuum line, if any. If traps must be placed on the floor, ensure they are in a secondary container to prevent breakage.
1. Waste Disposal and Cleaning Procedures
* Close or cover any open containers and leave the blower on for at least five minutes without any activity to purge the cabinet.
* Remove the panel and grill from the BSC and set them aside.
* After completing the work, decontaminate all equipment and supplies from the BSC by spraying them with the recommended disinfectant, 70% ethanol. Use an appropriate amount of ethanol and wipe clean the equipment, supplies, and BSC so no accumulation of ethanol occurs on the items.
* Never use the BSC for storing materials or equipment, as microorganisms may grow on residual culture media, and contaminate future experiments. Once the BSC is empty, disinfect it, and then close it.
* Turn off the blower and lamp.
* Replace the panel and grill, ensuring they are securely fastened.
* The UV lamp may be activated once the blower is off, and the viewing panel is fully closed.
* The UV lamp in the BSC may be cleaned using a lint-free cloth lightly dampened with alcohol weekly.
* Wash your hands and any exposed skin with soap and dry thoroughly after completing the clean-up procedure.

**5) Incident Reporting**

* Report any accidents resulting in injuries to the Principal Investigator and/or the departmental safety officer (DSO) immediately.
* For serious incidents, notify the Security Unit immediately by calling the 24-hour hotline on **23588999**.
1. **References**
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