






CAF Fluorescence Microscopy Unit
Laboratory Standard Operating Procedures

BIOSAFETY GUIDELINES FOR BSL-2 CONTAINMENT: BIOSAFETY CABINET II PROCEDURES			
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A. Purpose

This document serves as part of the Biosafety Manual and Standard Operating Procedures (SOPs) for the Central Analytical Facility (CAF) Fluorescence Microscopy (FM) Unit at the University of Stellenbosch, Room 2022-2025, Mike de Vries Building. It has been developed from earlier model Manuals and SOPs currently in place in the laboratory as well as Exposure Control SOPs, Safety Manuals, guidelines of the World Health Organisation and guidance of The Division of Occupational Health and Safety Office of Research Services, National Institute of Health (NIH).


All users of the CAF Fluorescence Microscopy Unit are required to fully understand the potential hazards involved in using these facilities and to always follow safety practices. Failure to do so can result in costly instrument damage and /or harm the user or cause serious injuries.

Use of the equipment is a privilege and not a right. No individual shall enter the facility or use any equipment without the approval of a CAF staff member. Training can be provided, however, it remains the discretion of CAF staff to allow independent use of any equipment.

This SOP must be considered together with all other SOPs of the unit.

B. Biosafety Cabinet (BSC) Procedures


1. The blower in the cabinet is to remain permanently switched on.
2. UV lights must be turned off before work begins in the cabinet. **Do not look directly at UV lights as this can cause eye damage.**
3. Check the certification sticker on the front of the unit to verify the biosafety cabinet's condition.
 - a. A qualified external contractor must annually certify these cabinets. If the re-certification date has passed, contact the Unit Manager.
4. Check the BSC air flow monitor to ensure proper functioning of the cabinet before placing any materials inside.
 - a. Readings indicate relative pressure drop across the HEPA filter. Higher readings may indicate filter clogging where a reading of zero may indicate loss of filter integrity. In either of these cases, notify the Unit Manager.
5. Disposable gloves and clean lab coats must always be worn when handling samples inside the cabinet. Gloved hands have to be sprayed with 70% ethanol every time they enter the BSC.


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6. The BSC should be decontaminated before work commences inside. Clean the inside surfaces of the BSC with 70% ethanol, 15 minutes prior to work being done.
7. DO NOT disrupt the airflow through the cabinet by placing ANY item in the front grills or by blocking the rear vents.
 - a. Disrupting the airflow into the front grill allows contaminated air from inside the cabinet to blow into the lab or directly at the person sitting at the cabinet. It also allows non-sterile air from the room to blow into the biosafety cabinet and over the experiments, increasing the risk of contamination.
8. Organize the work surface for a clean-to-dirty workflow. Place clean pipettes, flasks, and sterile media bottles at one side of the cabinet; place biohazard waste containers, used material, spent samples and other wastes on the other side.
9. While working, keep all material and perform work at least 4 inches away from the front opening of the cabinet, and minimize rapid movements or activity.
10. In general, the interior of the cabinet should be considered as a contaminated zone, even though every effort is made to keep the surfaces clean as per accepted good microbiological practices and sterile techniques.
11. Plastic serological pipettes with a cotton plug shall be used for all pipetting of liquids.
12. After handling potentially hazardous material in the cabinet, ensure all containers are closed tightly before removal from the BSC.

C. Decontamination during work in the BSCII

1. A beaker or discard pan, containing freshly prepared 1:10 solution of commercial bleach, shall be placed inside the biosafety cabinet to decontaminate all tips and serological pipettes.
2. After pipetting liquids containing BSL-2 compounds, the diluted bleach solution in the beaker shall be pipetted up and down the full length of the pipette before discarding the pipette.
3. Any other item such as cell culture flasks and plates that contained media with potentially hazardous material should be decontaminated. Add 10% bleach

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solution to these items, securely cap and ensure contact to the contaminated areas for 15 minutes at room temperature.

4. Any liquid waste should also be discarded into a waste beaker and decontaminated when work is has been completed. To deactivate pathogens, a 1:1 volume of freshly prepared 10% bleach solution should be added to the liquid and left at room temp for 15 minutes. Pour the liquid waste into a container which can be tightly closed (such as a used culture flask or falcon tube).
5. Discard all waste in the biohazard containers, including gloves, paper towels and all closed containers which have been in contact with biological material.
6. Plastic pipette tips and serological pipettes are treated as sharps and should be placed in a puncture resistant sharps container.

D. Disinfection after work in BSC II

1. After completion of work, clean the inside surfaces of the BSC with fresh 10% bleach solution and allow contact for 15 minutes.
2. Following the bleach decontamination, spray the surfaces with dH₂O and wipe away diluted bleach. Finally spray surfaces with 70% ethanol.
3. The UV light is turned on between procedures (at least 30 minutes but not more than 1 hour).
4. UV light is effective only for decontaminating clean, solid surfaces with which it comes into contact. It is not effective in decontaminating the cabinet air flow. UV light is not effective against bacterial spores. UV germicidal light tubes should be replaced frequently (at least every 6 months for biosafety cabinets used daily) to assure that they are emitting light at 254 nm and at an intensity appropriate for decontamination.
5. NOTE: Any use of volatile solvents, such as absolute ethanol, should be kept to a minimum or rather prepare the solution in a fume hood. Dangerously high levels of volatile vapours can accumulate inside the cabinet and pose a threat of fire or explosion.