CAF Fluorescence Microscopy Unit

Laboratory Standard Operating Procedures

BIOSAFETY GUIDELINES FOR BSL-2 CONTAINMENT: BIOLOGICAL SAMPLE HANDLING AND ANALYSIS			
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A. Purpose

This document serves as 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, Exposure Control SOPs, Safety Manuals and SOPs developed at other Departments of Stellenbosch University, from the University of Cape Town and guidelines of the World Health Organisation.

All users of the FM unit are required to fully understand the potential hazards involved in using these facilities and to follow safety practices at all times. Failure to do so can result in costly instrument damage and/or cause harm or seriously injure the user.

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 have to be considered together with all other SOPs of the unit.

B. Biological sample acceptance policy

1. Samples may only be delivered or brought into the unit after a risk assessment was performed and the appropriate sample submission form completed.

2. During risk assessment all potential routes of exposure (i.e. ingestion, inoculation, inhalation, skin and mucous membranes) needs to be identified. Most laboratory-acquired infections have resulted from cuts with sharps, inhalation of aerosols, splashes or sprays. If, during risk assessment, a particular agent is recognised as having a high hazard risk upon possible exposure, a safer and alternative procedure needs to be identified or other risk mitigation strategies applied.

3. Risk assessment of the biological samples itself should be performed where both the intrinsic hazardous properties of the samples as well as potential risk to individuals who manages the samples are considered. It should also address the potential for emergent hazards. The risks upon exposure to such samples may include:

    - Infectivity or ability to cause disease in a susceptible human host.
    - Severe outcome of the disease
    - Limited availability of preventative measures

    Availability of effective treatment is dependent on

- How the agent is transmitted (i.e. route of exposure)
- Quantity, concentration, and total volume used
- Stability in the environment
- Zoonotic concerns
- Allergenicity

4. The possible sources of exposure in the laboratory that poses a hazard risk i.e infection of researchers or the public, during the processing of biological agents, should also be evaluated during a risk assessment and these may include the following:

   a. Aerosol generation (e.g. pipetting, mixing, blending, sonicating, vortexing, shaking and cell sorting)

   b. Manipulation with sharps, including blades, sharp tweezers, or broken glass.

   c. Contact with blood, bodily fluids, or other potentially infectious material.

   d. Ingestion of agents via contaminated work areas

   e. Eye-splashes from liquid nitrogen during cryopreservation procedures

5. Samples are classified according to their potential level of hazard into 4 categories . It is the policy of the unit to only accept samples in the hazard group 1 or 2.

Hazard Group 1	Hazard Group 2	Hazard Group 3	Hazard Group 4
• Unlikely to cause disease	• Can cause human disease • May be a hazard to employees • Unlikely to spread to the community • Usually effective prophylaxis or treatment available	• Can cause severe human disease • May be a serious hazard to employees • May spread to the community • Usually effective prophylaxis or treatment available	• Causes severe human disease • Serious hazard to employees • Likely to spread to the community • Usually no effective prophylaxis or treatment available

6. No samples with known infectious material or any material from hazard group 3 or 4 will be allowed in the unit. Should such samples need to be analysed, it is required that the

samples are adequately inactivated for example through fixation with appropriate fixative (4% formaldehyde, methanol, ethanol or glutaraldehyde) in order for those samples to be considered as hazard group 1 or 2.

7. All Genetically Modified Organism (GMO) samples must be declared in the sample submission form completed prior to the samples reaching the laboratory. Documentation should also be submitted that the laboratory where the GMOs originated, is a registered GMO facility or has a pending application.

8. All GMO samples are classified as hazardous and categorised based on the risk potential to human health and wellbeing as well as the environmental effect should the samples be released into the environment.

    a. GMO Risk Group 1:

        - Agents posing low individual and community risk.
        - A biological agent that is unlikely to cause disease in healthy workers or animals.

    b. GMO Risk Group 2:

        - Agents posing moderate individual risk and limited community risk.
        - An agent (pathogen) that can cause human or animal diseases but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures to these agents rarely cause infection leading to serious disease; and effective treatment and preventative measures are available.
        - The risk of spread of these agents is limited.

    c. GMO Risk Group 3:

        - Agents posing a high individual risk but low community risk.
        - A pathogen that usually causes serious human or animal disease, or which can result in serious economic consequences.
        - A pathogen that does not ordinarily spread by casual contact from one individual to another.
        - Pathogens that can be treated by anti-microbial or anti-parasitic agents.

d. GMO Risk Group 4

- Agents posing a high individual risk and high community risk.
- A pathogen that usually produces very serious human or animal disease, often untreatable.
- A pathogen that may be readily transmitted from one individual to another, or from animal to human or vice-versa directly or indirectly, or by casual contact.

9. As a biosafety level 2 facility the laboratory only offers level 2 containment, so no samples in GMO risk group 3 or 4 may be submitted to the laboratory.

10. Staff processing the samples need to notify yhe unit manager on any health concerns, such as immunodeficiency, pregnancy, allergies etc.

C. Nature of sample material

1. A diverse set of samples are submitted for analyses to the FM unit which may include micro-organisms such as yeast, fungi and bacteria with low risk of infecting to the users or staff of the unit. In addition, different cell lines, including human derived or other mammalian cell lines as well as primary cells isolated from mammals or humans are also analysed at the unit.

2. Plant samples (e.g. leaves, flowers, stems, roots, seeds and fruit) can be analysed at the unit and submitted either fresh, fixed or frozen. These samples can be submitted already prepared on microscope slides, ready for imaging or can be prepared for analyses by staff in the unit.

3. Plant samples send for analyses may be infected with fungi or other infectants

4. Heat-inactivated or fixative treated samples are regarded as low-risk samples.

5. All biosafety hazard level 2 samples as well as GMO samples are regarded as high-risk samples and needs to be processed in the BSC II cabinet. These includes:

- Live mammalian cell cultures, either primary or commercial cell lines

- Live bacteria (or other micro-organisms) in media could be unintentionally contaminated with infectious agents. Cross-contamination between users' needs to be considered as a possibility.

- Human tissue samples such as blood

D. Arrival of samples to the unit

1. Where possible, samples should be prepared in the user's own laboratory and only an appropriate volume required for analyses should reach the FM unit.

2. The container in which the samples are kept should be labelled with the user's name and surname, description of contents, date and biosafety level as well as the pre-analysis storage (short-term) instructions and post-analysis sample procedure.

3. Dangerous goods and biological materials should be packaged securely to contain the content, prevent accidental exposure or substances leaking during transport to protect people and the environment. Transport regulations, packaging and additional labelling guidelines will depend on the classification of the material to be transported.

4. In some cases, it is acceptable to perform certain steps of preparation in the unit. These samples need to be processed and managed according to their hazard level which is either level 1 or level 2

5. Unless samples need to be analysed unfixed e.g., dynamic processes or viability assays, samples should be fixed appropriately prior to arrival.

6. If samples need to reach the unit live or fresh for analyses, they need to be maintained and processed according to their risk level.

7. Culture plates with live cells should be secured with parafilm when they reach the unit and will also be analysed in this format.

8. All GMO samples are regarded as hazardous samples, irrespective of the species of the samples.

E. Sample transfer to flow cytometry tubes, microscope slides or microscope dishes

1. When managing samples in the unit, the correct PPE should be worn which includes gloves and a lab coat, closed shoes and clothing which prevent skin exposure.

2. Users who will be participating in the analyses need to bring their own clean lab coats to the unit.
3. Gloves (and face masks where required) will be provided by the unit.

E.1. Transfer of non-hazardous micro-organisms

1. Non-hazardous micro-organisms or fixed samples may be opened on the bench and transferred with calibrated pipettes into flow cytometry tubes, onto microscope slides or into imaging dishes.

2. If the samples tend to clot easily, they need to be filtered using a 50µm mesh filter during transfer to the flow cytometry tubes.

3. Sample tubes should be capped if they are not going to be analysed immediately after transfer to the appropriate sample holder.

4. A cover slip should be placed on top of samples mounted onto microscope slides.

5. The lid of an imaging dish should always be on the dish during the experiment.

E.2. Transfer of BSL 2 level hazardous samples or samples classified as GMO samples

1. All hazardous samples should be kept in airtight containers or tubes in the unit.

2. The uncapping of BSL 2 samples need to be performed in the BSCII safety cabinet in room 2022. The further processing of these samples will continue in the BSCII safety cabinet, strictly following the procedures at set out in the SOP of the BSC II safety cabinet (see SOP6).

3. Before any samples are removed from the BSCII cabinet, they need to be closed again and the surfaces of the cabinet disinfected with 70% ethanol.

F. Sample handling during an analysis or imaging session

F.1. Flow Cytometry and cell sorting

1. All fixed samples and low risk samples may be kept on the bench in uncapped tubes for the duration of the experiment.

2. Live samples and biohazardous samples should always be kept in capped flow cytometry tubes and the cap only removed prior to placing the tube in the sample loading port.

3. Operation of the flow cytometer should be done according to procedures described in the instrument manual and as instructed by the unit staff.

4. Immediately post acquisition, the tube needs to be removed and capped.

5. After cell sorting, the sort collection device used such as a tube or a plate needs to be capped tightly or covered with the appropriate lid, directly after removal of the device from the cell sorter.

6. If any other procedure is required to be performed on the sorted samples, they need to be opened only inside the BSCII cabinet.

7. Culture plates used to collect sorted samples need to be sealed with parafilm post-sorting.

8. When samples from hazard group 2 is sorted and the production of aerosols poses a risk of infection, the Aerosol Management Unit of the flow cytometer should be used according to the instructions of the manufacturer.

F.2. Fluorescence Microscopy

1. Samples prepared on slides and ready for analyses can be placed onto the microscope slide as per normal procedure.

2. Samples for live cell imaging, need to reach the unit in the correct imaging dish with its lid securely in place at all times.

3. Samples classified as low risk may be opened and processed on the bench.

4. Samples with a biosafety risk level of 2 need to arrive at the microscope, sealed and ready to be placed on the microscope stage. Only when treatment of the sample is required during the imaging session, may the samples be opened in the BSC II cabinet.

5. Operation of the microscope should be done according to the manual provided and as instructed by the FM unit staff during training

6. When using the on-stage incubator of the microscope

    a. The CO_2 supply should be opened at the gas regulator attached to the CO_2 tank.

b. The gas and temperature control units should be switched on approximately 10 minutes prior to the start of the experiment.

c. The incubator should be secured in place on the stage before water is added to the water trough to maintain humidity.

d. If an oil objective is to be used, a droplet of the immersion oil should be put onto the objective, followed by placing the sample holder in position inside the incubator.

e. Ensure that the lid of the incubator is securely in position.

f. To change oil on the objective, the sample holder needs to be carefully removed and the objective wiped in one direction with lens cleaning tissue ONLY. Place a fresh droplet of oil on the objective and return the samples holder securely back into position.

G. Post-Analysis cleaning

G.1. Cleaning workspaces and equipment

1. All work surfaces need to be cleaned with 70% ethanol. The keyboard and mouse should be wiped with a disposable tissue paper dampened with 70% ethanol.

2. The flow cytometer should be cleaned according to the standard cleaning protocol:

    a. Run 5 minutes of 70% ethanol, followed by 5 minutes of 10% bleach and finally 5 minutes of distilled water.

3. The microscope stage insert and any other parts exposed to samples should be cleaned with 70% ethanol.

G.2. Waste disposal

All samples and other contaminated waste should be discarded as described in SOP3.

In short:

1. All live samples should be inactivated by adding an equal volume of 10% bleach to the sample.

2. All contaminated waste, residual samples, gloves, tissue paper and used sample tubes should be placed in a plastic bag to be sealed and labelled with name of the user, date and contents.

3. The plastic bag should then be placed in the red lined biosafety bin in the unit.

4. All plastic serological pipettes, blades, microscope slides, coverslips and any other sharp objects should be placed in designated yellow sharps bins.

5. Liquid waste must be disinfected with 10% bleach for at least 10 minutes and could then be discarded into the laboratory sink in the Molecular Laboratory of the Department of Physiology, room 2026.