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CAF Fluorescence Microscopy Unit

Laboratory Standard Operating Procedures

BIOSAFETY GUIDELINES FOR BSL-2 CONTAINMENT: NANOMATERIAL HANDLING AND ANALYSIS			
SOP#: 5	Version: 1	Effective Date: 01 June 2021	Page **1** of **7**

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2020-11-08	Date: 2021-06-01	Date: 2021-06-07
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Content:

A. Purpose

This document serves as part of the Biosafety Manual and Standard Operating Procedures for the Central Analytical Facility (CAF) Fluorescence Microscopy Unit at the University of Stellenbosch, Room 2022-2025, Mike de Vries Building. It has been developed from earlier model Manuals and Standard Operating Procedures (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, NIH.

All users of the CAF Fluorescence Microscopy 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, serious injuries or harm.

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. Sample acceptance policy

1. Nanoparticles and other non-biological samples developed at Stellenbosch University and other research institutions can be sent to the CAF Fluorescence Microscopy Unit for analyses.

2. Samples may only be delivered to the unit or brought into the unit after a risk assessment was performed and the appropriate sample submission form completed: https://forms.gle/UgMpbCmsgQvan1iA8.

3. When performing a risk assessment of laboratory procedures, all potential routes of exposure should be evaluated. Most laboratory-acquired infections have resulted from cuts with sharps, inhalation of aerosols, splashes or sprays, hence, it is good practice to look for potential exposures via main routes of entry such as ingestion, inoculation, inhalation, and contamination of skin and mucous membranes. If high risk for exposure is defined during the assessment, a safer alternative procedure needs to be identified or other risk mitigation strategies applied.

4. Depending on the potential risk involved, samples are categorised into four biohazard levels. It is the policy of the unit to only accept samples in hazard group 1 or 2. Refer to the Table 1.

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

Table 1: The four biohazard risk categories

5. No samples with known infectious material or any material posing serious risk to occupants of the facility (such as those in hazard group 3 or 4) are allowed in the unit.

   Staff members, or users handling samples, needs to notify the unit manager of any health concerns, such as immunodeficiency, pregnancy, allergies etc. before processing any samples

C. Risks involved with handling nanomaterials

1. Studies have indicated that because of their small size, nanoparticles:

   a. Are more likely to deposit in the respiratory tract,

   b. Can penetrate across cell membranes,

   c. May be biologically active,

   d. Can persist in tissue, leading to delayed toxicity.

2. Nanoparticles which are either used in powder or liquid format and any form of processing that may increase the likelihood of nanoparticles to become airborne, is a health risk. These may include: pouring and/or mixing, cleaning up spills, aerosolizing nanoparticles through vortexing or other procedures.

3. The effects of nanoparticles are highly variable and depends on the product type, concentration used and route of entry or exposure. Always read the labels and MSDS/ SDS

of the specific products used to identify their hazards and potential toxic effects and communicate the information to the unit staff prior to sample submission.

4. As the nature of the material may vary, it should not put the user or unit staff at unnecessary risk. Material should be stable during analysis.

5. In assays where the nanomaterial may be combined with biological samples such as yeast, fungi and bacteria or mammalian cell lines (Refer to SOP4 for handling of biological material), the risks involved with the handling of the samples need to be evaluated based on both the non-biological as well as the biological sample.

D. Arrival of samples to the unit

1. Samples should be prepared in the user's own laboratory and only an appropriate volume required for analyses should arrive at the unit. Further handling is restricted to the transfer of samples to the appropriate sample holder required for microscopy or flow cytometric analyses at the FM unit.

2. The container in which all the sample are kept should be labelled with the user's name and surname, description of contents, date, biosafety level, pre-analysis storage (short-term) instructions and post-analysis procedures. The samples need to display the necessary hazard labels where appropriate.

3. Dangerous goods and biological materials must be safely packaged to contain and prevent accidental exposure or release of substances during transport. It should be packed in airtight primary and secondary packaging and where necessary also tertiary containers.

4. In live cell imaging of nanomaterials, samples need to always arrive at the unit in the correct imaging dish with its lid in place. Culture plates with live cells should be secured with parafilm when they arrive in the unit and should be kept this way until the end of the experiment.

E. Sample transfer to microscope slides or microscope dishes

1. When handling nanomaterial in the unit, the correct PPE should be worn which includes gloves and lab coats and if material is in powder form or there is any risk of becoming airborne, a face mask and eye protection.

2. Users who are present in the unit during the analyses need to bring their own clean lab coats to the unit.

3. Gloves (face masks and eye protection where required) will be provided by the unit.

4. When samples are transferred to the appropriate sample holder, they need to be handled in the Fume Extraction Cabinet in room 2022

5. Before any samples are removed from the cabinet, the containers need to be tightly closed.

F. Safe use of the fume extraction cabinet

1. Always use double gloves when working in the hood, as well as a laboratory coat that covers the arms up to the gloves. Sleeve covers can be worn over the sleeves should any skin be exposed.

2. Do not lift the safety glass higher than breast height or as indicated by the manufacturer, to avoid direct exposure to the face.

3. The airflow in the hood should never be switched off, to avoid toxic fumes leaking into the laboratory and affecting other users.

4. Only discard waste in the designated and appropriately labelled waste bottle. If any non-specified reagent is to be discarded, it needs to be clearly communicated to the CAF staff who will issue the user with a designated waste container to be placed in the hood. The contents of such reagents and mixes must be communicated with the CAF staff so the required procedure can be followed when waste is removed from the facility.

5. Do not overfill waste bottles and notify CAF staff if any containers need to be emptied.

6. All users of the safety hood are required to read and sign these documents, even if they are accompanied and trained by a senior student/researcher.

7. All users need to familiarise themselves with the risks involved with the reagents they use by reading the MSDS files on each reagent.

8. No unlabelled sample holders or reagent bottles are to be left in the hood. A name, date, contents and potential hazard need to be clearly indicated. Also indicate if the samples need to remain in the hood for longer than 24 hours, else the CAF staff will discard samples and reagents after this time.

9. Do not overfill the hood and place bottles to the side of the hood to ensure proper air flow at the back of the hood.

10. The area needs to be left clean and tidy once a user has finished working in the hood

G. Post-Analysis procedures

G.1. Cleaning, spills and exposure

1. All work surfaces, including the computer mouse and keyboard, need to be cleaned with dampened tissue paper and dried afterwards with absorbent paper towels.

2. The microscope stage insert and any other parts exposed to samples should be cleaned with tissue paper dampened with distilled water and dried with absorbent paper towels.

3. In case of a spill, place absorbent tissue paper over liquid spills and wet tissue paper over powder spills. Follow the SOP on spills and exposure (SOP2).

4. For large spills of nanomaterial, notify Facility Management and alert the Fire Department.

G.2. Waste disposal

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

In short:

1. Waste containing nanoparticles should be kept in secure containers and dealt with as a chemical waste.

2. Samples, contaminated gloves, bench paper and clean up materials should be double bagged, closed tightly and labelled as “nanoparticle waste.”

3. The bag containing the chemical waste should be labelled with details on the content: name of the nanomaterial, approximate concentrations of constituents as well as hazard label where required.

4. The bag should be placed in the green chemical waste bin for solid chemical waste in room 2022, next to the fume extraction cabinet.