




**CAF Fluorescence Microscopy Unit**

**Laboratory Standard Operating Procedures**

<b>BIOSAFETY GUIDELINES FOR BSL-2 CONTAINMENT: WASTE DISPOSAL</b>			
SOP#: 3	Version: 2	Effective Date: 1 June 2021	Pages: 1 of 9
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## **A. Purpose**

This document serves as a 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 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 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.

## **B. Nature of sample material to be discarded.**

The variety of samples analysed at the CAF Fluorescence Microscopy Unit by flow cytometry, cell sorting or confocal microscopy may include mammalian cell lines, yeast, fungi, blood (fish and human), bacteria or environmental samples from soil, compost, bioreactors etc.

Patient blood submitted for immunological assessment or animal blood used for ploidy analysis from species such as catfish and tilapia fish.

Various plants tissue samples such as fruit, leaves, stems etc. for dissection and freezing for cryo-sectioning and imaging at a later stage. In some cases, these are infected with fungi or other infectious agents.

Blood smears and other human and animal tissues already prepared on microscope slides to be visualised with the microscope. Where tissue has been frozen in liquid nitrogen, samples might be sectioned on the cryostat in the unit to prepare the microscope slides for imaging.

The super-resolution microscope is often used to visualise nanoparticles to be used for cellular uptake and devices designed by the engineering faculty for imaging.

The samples can be provided in the following formats i) fixed, of which the fixative reagent could include either 4% formaldehyde, methanol, ethanol or Formaldehyde Alcohol Acetic Acid (FAA); ii) fresh: e.g. plant material; iii) live cell cultures: e.g. mammalian cell lines and microorganisms or iv) frozen, such as samples for cryo-sectioning which may include biofilm, animal, human or plant tissue samples.

All biological and nanomaterials are discarded in the same way irrespective of their biohazard level.

Some biological samples, submitted to the unit, may have been genetically modified to express specific fluorescence proteins. These samples should be treated according to the GMO Act. (<https://www.gov.za/documents/genetically-modified-organisms-act-0>)

### C. Containers for waste disposal

#### *C.1. Containers designated for biohazardous waste.*

1. The red bin, also identifiable by the Universal Biohazard symbol printed on the side, is used to discard biohazard waste.
2. It can be found in room 2022 next to the Biosafety II cabinet
3. These containers are solid, leak proof and lined with red biohazard bags.
4. The container lid should be kept closed unless someone is working nearby and regularly adds waste to the container.
5. When the red bag is approximately  $\frac{3}{4}$  full, the bag must be closed with a zip tie and the lid secured placed (the lid locks into place) on the waste container.



### ***C.2. Sharps disposal containers***

1. Yellow sharps disposal containers are available in each room in the unit and can be further identified by the Universal Biohazard symbol printed on the side.
2. These sharps disposal containers are made from rigid plastic. The outside of the container is clearly marked with a red line that indicates when the container should be considered full and needs to be disposed.
3. Sharps containers should never be overfilled and closed when the contents reaches the red line which is about 3/4 the volume of the container.



### ***C.3. Ultimate form and destination of inactivated biological waste***

1. Containers and liners are labelled in compliance with SANS 10248-1. They are labelled “Warning/Danger” in 3 languages, display the International bio-hazardous label, UN and class 6.2 numbers, as well as specific notification of the type of waste.
2. When container lids are secured, the bins need to be taken to the dedicated area for biohazardous waste storage of the Molecular Laboratory, room 2026, Department of Physiological Sciences, Mike de Vries building, Stellenbosch University.
3. Filled biological waste containers and sharp containers are collected by the company: BCL Medical Waste Management.
4. A biological waste pick-up request form needs to be completed (Refer to Appendix 1) and send to BCL Medical waste Management ([orders@bclmedicalwaste.co.za](mailto:orders@bclmedicalwaste.co.za)) to initiate the collection and disposal of the filled biohazard/sharp containers. If indicated on the request form, these bins will be replaced with empty containers.
5. The biowaste company has a fleet of specialised vehicles dedicated for transportation of biomedical waste. The vehicles are registered with the City of Cape Town in line with the Environmental Health by Law to transport medical waste. Drivers all hold code 10 licences endorsed with the Dangerous Goods Permit and annual training on the transportation and handling of hazardous waste is provided to all drivers and assistants. Disposal of all categories of Health Care Risk Waste (HCRW) is by high temperature incineration.

**C.4. Containers for chemical waste**

1. Green chemical waste bins are available next to the fume extraction cabinet.
2. All contents of chemical waste need to be disclosed. This information needs to be clearly visible on the label of the bottle or holder containing the chemical.
3. A waste profile sheet needs to be completed with this information and sent to the contractor, EnviroServ, for approval and potential testing prior to collection. Collection is arranged per request form and per the company's schedule for picking up from Stellenbosch University.



**C.5. Bin for regular waste**

1. A waste bin for normal municipal waste is available at the entrance of the unit. The bin is only for municipal waste and is removed with the waste of the Department of Physiological Sciences.
2. The bin is cleaned and decontaminated regularly.

**D. Disposal procedures for various material**

**D.1. Solid biohazardous waste and residual samples**

1. In the case of live organisms analysed, all residual samples need to be inactivated by adding 70% ethanol to the sample tube or culture plate.
2. All tubes containing samples need to be capped airtight before discarding and culture plates sealed with tape or parafilm to prevent leakage.
3. The tubes/plates need to be transferred to plastic waste bags provided by the facility and sealed to contain all contents and prevent leakage. The waste bags need to be labelled using a permanent marker with the user's name, date and the type of waste. These bags need to be tightly sealed and securely placed in the allocated biohazard red waste bin.
4. Gloves and any potentially contaminated material such as tissue paper used to clean work areas and empty sample holders etc. should be discarded into red biosafety bins.

### ***D.2. Contaminated liquids***

1. Blood, aspirated tissue culture media, or other liquid waste generated from BSL2 experiments must be disinfected and then disposed. Bleach is typically used to disinfect liquids.
2. If bleach is used:
  - a. The final concentration should be 10% sodium hypochlorite (no less than one part bleach to 9 parts liquid).
  - b. The bleach should be freshly prepared
  - c. Disinfect contaminated liquids for at least 15 minutes prior to disposal.
  - d. Dispose the liquid down the sink.
3. When an empty waste tank is connected to the flow cytometer, 2 Medisure sachets of 6g chlorine needs to be added to the tank as disinfectant before the waste fluid is collected. When the waste bottle is full, the contents need to be discarded in the laboratory sink in the Molecular Laboratory, room 2026.
4. If any container with contaminated fluid will be unattended for a short period of time due to a specific and valid reason, label it as "Biohazardous liquid" with the date and time and if possible store in a secondary container (for example, a plastic tub with a lid) during the time of disinfecting.

### ***D.3. Contaminated glass and other sharps***

1. All contaminated glassware, such as microscope slides, cover slips and glass pipettes must be carefully disposed of in allocated yellow sharps disposable bins.
2. Sharps should never be discarded into regular trash or into the bags meant for biological waste.
3. Disposable plastic pipette tips are considered puncture hazards and should also be discarded in the dedicated sharps bins.
4. No tissue paper or gloves should be discarded in these bins.

5. Blades used for dissections or cryo-sectioning should be placed in the sharps bins immediately after use.
6. Broken glassware that may be contaminated needs to be collected using a mechanical device such as tongs, forceps or a brush and dustpan and placed in the glass disposal bin of the Molecular Laboratory (room 2026), Department of Physiological Sciences. Goggles should be worn whilst collecting small pieces of broken glass when using a dustpin and pan to prevent contact of airborne dust glass with eyes.

#### ***D.4. GMO samples***

All GMO samples should be regarded as biological hazardous material and should be discarded according to the procedures above.



**Appendix 1: Example of Waste Removal Request form**

**US Risk & Protection Services**

**CHEMICAL / BIOLOGICAL WASTE PICK-UP REQUEST FORM**

Date: \_\_\_\_\_

**Department Information**

To: **BCL Medical Waste**

From: **Me Lize Engelbrecht**

Building: **Mike de Vries Building**

Department: **Physiological Sciences, Stellenbosch University**

Room #: **2022**

Tel: **021 808 3515**

Mobile: **xxx xxx xxxx**

E-mail: **lizeb@sun.ac.za**

**WASTE INFORMATION**

Please arrange for the disposal of the following hazardous waste material:

Container Code  (Note 1)	Type of Waste / Waste Description  (Note 2)	Quantity to be removed with size of container  (Note 3)	Quantity to be replaced with size of container.  (Note 4)	Other Comments  (Note 5)
	Biomedical waste	4 containers of 70L re-usable bin	10 containers of 70L re-usable bin	Collect/Replace
	Biomedical waste		10 <u>only liners</u> for 70L re-usable bin	Replace
	Biomedical waste	3 containers of 20L yellow bin	15 containers of 20L yellow bin	Collect/Replace
	Biomedical waste	10 containers of 7.6 L Yellow sharps bin	10 containers of 7.6 L Yellow sharps bin	Collect/Replace
	Biomedical waste	9 containers of 50L box with liner	15 containers of 50L box with liner	Collect/Replace
	Biomedical waste	0 containers of 25ℓ plastic bottle	0 containers of 25ℓ plastic bottle	
<b>Biohazards tape</b>			0 roles of	
<b>Cable Ties / 100</b>			0 bags of	

Notes:

1. Container code refers to the numbers as allocated by Waste-Tech / Sanumed, e.g. 1A or 11C, Medical Box, etc. Remember that Sanumed does not allocated numbers to the different departments.
2. Type of waste refers to whether it is Radioactive, Chemical, Biological or Medical waste.
3. Quantity to be removed refers to number of containers to be removed and the size of the containers.
4. Quantity to be replaced refers to the number of containers to be replaced and the size of the containers.
5. Other comments refer to whether the containers are a medical box or a metal container or whether you require a smaller or larger container.
6. Remember: If no containers are requested, none will be delivered.