

**SAFETY MANUAL  
AND  
STANDARD OPERATING PROCEDURES**

**FOR THE  
CFAR Flow Cytometry Core**

**NEW YORK UNIVERSITY  
SCHOOL OF MEDICINE**

**Old Bellevue C&D building, Room 645**

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## Table of Contents

List of Key Personnel .....	1
Important Telephone Numbers .....	1
CFAR Flow Cytometry Core Laboratory Layout .....	2
1. Background .....	3
1.1 General Information about HIV .....	3
2. Risk to Lab Personnel.....	3
2.1 Definition of Biohazardous Specimens.....	3
2.2 High-speed Cell Sorting.....	3
3. Containment.....	4
3.1 Facility Layout .....	4
3.2 Laboratory Facilities.....	4
3.3 Biosafety Cabinets and Aerosol Management Unit .....	4
3.4 Biohazard Labels .....	5
4. Facility Entry and Exit.....	5
5. Training.....	5
6. Medical Requirements, Surveillance, and Responses to Exposure ...	5
6.1 Medical Requirements.....	5
6.2 Medical Precautions and Surveillance .....	5
6.3 Medical Response to Exposure.....	5
7. Standard Operating Procedures for the CFAR Flow Cytometry Core .....	6
Standard Operating Procedures	
FLOW-101 Standard Laboratory Practices for the CFAR Core .....	7
FLOW-102 Spill Response and Reporting.....	14
FLOW-103 Exposure Incidents and Reporting .....	15
FLOW-104 Shipping and Receiving Infectious Substances and On-campus Transportation of Biologicals Samples .....	17
FLOW-105 Medical and Facility Emergencies .....	18
BSL-2 Sorting User Approval.....	20
Core Facility Spill / Accident Report.....	21

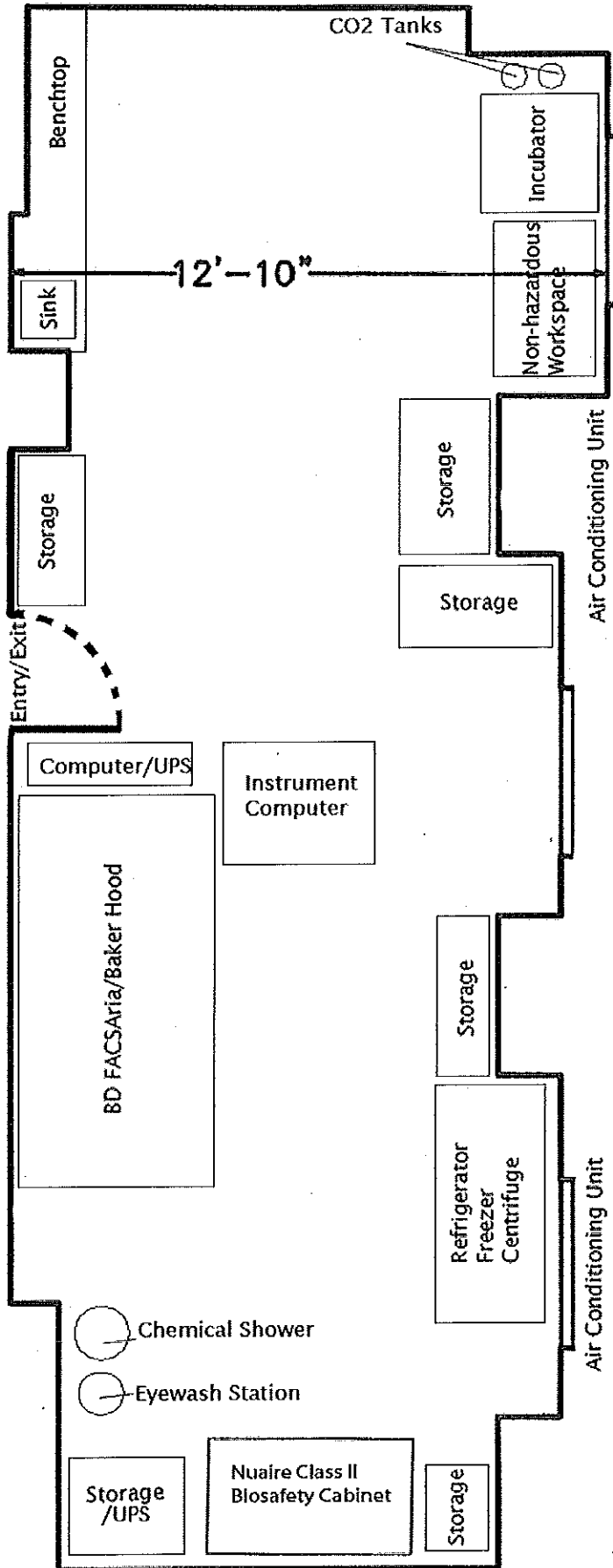
## List of Key Personnel

Name	Title	Extension	Mobile
Derya Unutmaz	Co-Director	x39203	
Peter Lopez	Co-Director	x30635	646-469-3399
Michael Gregory	Sr. Lab Tech/Lab Manager	x35063	516-641-5185
Keith Kobylarz	Lab Technician	x35907	718-664-8633
Kamilah Ryan	Lab Technician	x35907	917-715-5337
Nicole Hanson	Lab Technician	x35907	520-465-2001

## Important Telephone Numbers

<i>Note: In an emergency, Communications can page personnel from key departments</i>	
Any Medical Center Emergency	x33911
Building Services	x35071
Communications	x37403
NYULMC Emergency Room (ER)	x35550
Bellevue Emergency Department (ED)	(212) 572-5082
Employee Health Services (EHS)	x35120
Environmental Health and Safety (EH&S)	x35159
Ginelle Andrews, Biosafety Specialist	x35971
Gerry Griffin, Associate Director	x36944
NYULMC Facilities Management	x35275
Bellevue Facilities Management	(212) 562-4779
Poison Control	(212) 764-7667
Radiation Safety	x36888
Security	x73000

# HIV Core Laboratory Layout



## 1. Background

### 1.1. General Information about HIV

Human immunodeficiency virus (HIV) is a retrovirus that causes *acquired immunodeficiency syndrome* (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections.

Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. The three major routes of transmission are unprotected sexual intercourse, contaminated needles, and transmission from an infected mother to her baby at birth, or through breast milk.

HIV primarily infects vital cells in the human immune system such as helper T cells (specifically CD4<sup>+</sup> T cells), macrophages and dendritic cells. HIV infection leads to low levels of CD4<sup>+</sup> T cells and eventual susceptibility to opportunistic infections that result in AIDS and death.

## 2. Risk to Lab Personnel

### 2.1. Definition of Biohazardous Specimens

All unfixed human and primate cell suspensions and tissues must be treated as potentially infectious, and handled in accordance with universal precautions for blood borne pathogens (i.e., handle as if infected with HIV, HBV, HCV etc.). This applies to cultured cell lines as well as primary tissue suspensions (e.g., blood, bone marrow, cells derived from solid organs). It also applies to nonhuman cells that have been deliberately infected with known or potential human pathogens. Although standard BSL-2 working conditions are usually acceptable for handling such specimens, the potential of cell sorters to generate high levels of aerosolized microdroplets require a higher level of containment. For the purposes of high speed cell sorting, specimens considered to be potentially biohazardous include all of the following:

- Suspensions of primary human or primate cells from blood or other tissues.
- Cultured and in vitro passaged human or primate cell lines. Note that with few if any exceptions, established human cell lines may fall into the “potentially biohazardous” category, and therefore cannot be sorted unless specific recommendations for sorting biohazardous specimens are followed.
- Primary cells or cell lines that have been transformed with an immortalization agent that has the potential to transform human cells, such as Epstein-Barr virus or a potentially oncogenic retrovirus or lentivirus.
- Any samples known to contain or have been exposed to infectious pathogens normally handled at BSL-2 conditions. This includes agents such as viruses (HIV, HCV, HBV, CMV, EBV, influenza, etc.), bacteria (*Listeria*, BCG and other attenuated mycobacteria, staphylococci, streptococci, various Gram negative pathogens, etc.), fungi (*Cryptococcus*, *histoplasma*, *aspergillus*) and protozoa (*Toxoplasma*, some plasmodia, cryptosporidia, etc.).

### 2.2. High-speed Cell Sorting

High speed droplet based cell sorters can generate large amounts of aerosols, and recently

published standards now specify a much higher level of biocontainment for cell sorting of unfixed human cells or other potentially biohazardous samples than have been traditionally followed. "If aerosol containment is incomplete, the safety features of the cell sorter must be modified such that no escape of aerosol can be detected. Alternately, sorters can be placed inside a biosafety containment cabinet" (Ref: I Schmid et al., International Society for Analytical Cytology Biosafety Standard for Sorting of Unfixed Cells. Cytometry Part A, 71A:414-437 (2007)).

Sorting of samples that represent potential toxic or infectious exposures via the aerosol route therefore require special procedures and laboratory conditions. This is true even for agents that are normally handled under standard BSL-2 laboratory conditions, such as primary human cell suspensions or cell lines. The heightened concern in the case of cell sorting arises from the possibility that cells or microorganisms may be delivered directly into the lungs of personnel in the vicinity of a cell sorter. In theory, this could increase the risk of infection with an occult pathogen, transfer of genetic material, sensitization to antigens or other potentially harmful effects. Although such adverse effects have not been documented as a consequence of exposure to aerosols during cell sorting, there is sufficient concern about this to warrant the implementation of procedures to eliminate any excess risk to personnel.

### 3. Containment

#### 3.1. Facility Layout

3.1.1. The CFAR Flow Cytometry Core Laboratory is a Biosafety Level 2 (BSL 2) certified facility that is located in Bellevue Hospital's C&D Building, room CD645.

3.1.2. The facility is cleaned and maintained by the laboratory staff.

#### 3.2. Laboratory Facilities

3.2.1. The laboratory has one sink for hand washing near the entrance to room CD645. An eye wash station and emergency shower are located at the south end of the room.

3.2.1.1. The eye wash station and emergency shower are maintained and inspected by the Bellevue Hospital Facilities Department (212-562-5106).

3.2.1.2. A second emergency shower is located in the hallway in front of room CD640. Bottles of eye wash solution are available across the hall in room CD647 (access code: 5603\*).

#### 3.3. Biosafety Cabinets (BSCs) and Aerosol Management Unit

3.3.1. There is one four foot Nuair Class II BSC, and one Baker BioProtect III Class II BSC located in the facility. The Baker BioProtect cabinet both houses the Aria Flow Cytometer and provides extra bench space for sample preparation.

Name/ Room#	Room	Model	BSC Serial No.
Baker BioProtect III BSC	CD645	BioProtect III	102073
Nuair BSC	CD645	Nu-425-400	14398 ST

3.3.2. **Certification of BSCs** - Environmental Health and Safety (EH&S) retains a vendor (Technical Safety Services, Inc. [www.techsafety.com](http://www.techsafety.com)) who certifies each BSC **annually**. The certification is conducted in accordance with NSF Standard 49 and currently accepted best practices.

**3.3.3. Whisper Aerosol Management Unit** –This unit is supplied by the FACSAria Cytometer manufacturer (BD Biosciences) to remove and filter aerosols from the cytometer’s interior compartments. The Whisper unit uses an ULPA filter that is changed every 6 months or if airflow indicator is below 3 inches WC. There is a second Whisper unit in the lab which can be used as a spare.

#### **3.4. Biohazard Labels**

All equipment used for storage of infectious agents must have biohazard labels specifying the agent(s) stored.

### **4. Facility Entry and Exit**

#### **4.1. Refer to SOP FLOW-101**

### **5. Training**

Prior to being allowed independent access to or performing work independently in the facility, all personnel will be trained by an approved lab user and must be approved by a Co-Director of the CFAR Flow Cytometry Core.

Training will include knowledge of the Safety Manual and approved protocols, followed by observation of a certified user performing the intended procedures. Then the trainee will work under supervision of a certified lab user until the certified user gives approval and has successfully completed all training requirements outlined in Form FLOW-101F (*BSL-2 Sorting User Approval*). A copy is at the end of this manual), for the new user to be certified to enter the core or work independently in the facility.

### **6. Medical Requirements, Surveillance, and Responses to Exposure**

#### **6.1. Medical Requirements**

Workers with a known immunodeficiency disease or who are taking immunosuppressive medications are not permitted to work in the virus room without prior approval by the VCT Core Laboratory Director, the Biosafety Officer/Specialist, and Employee Health Services (EHS). Workers with open wounds that cannot be adequately covered cannot work in the virus room. EHS can provide medical advice to workers who are not sure whether they fall into any of these exclusionary categories.

#### **6.2. Medical Precautions and Surveillance**

All NYULMC personnel working with patient samples are offered a Hepatitis B (HBV) vaccination at their Employee Health Screening. Personnel who intend to work with BSL-2 bloodborne pathogens or other potentially infectious materials, samples can obtain an HBV immunization by contacting EHS at 212.263.5020. The Employee Health Center can also test whether an HBV immunization is still effective.

Baseline HIV testing is required before working with biohazardous HIV-infected samples. HIV testing can be obtained at the NYULM Employee Health Center by contacting them at 212.263.5020. HIV testing is required subsequently in cases of accidental or suspected exposure. Personnel are also encouraged to speak to their primary care physicians about regular HIV testing.

Non-NYULMC employees are responsible for maintenance of their HBV immunizations and for their own HIV testing.

#### **6.3. Medical Response to Exposure**



Procedures for management of exposure due to cuts are detailed in Safety Policy 135, *Bloodborne Pathogens Exposure Control Program*. These procedures apply to all NYULMC employees and as such apply to all employees working in the CFAR Flow Cytometry Core. These procedures are stated below.

All cuts and other exposures to blood or other body fluids must be reported *immediately* to EHS, or if EHS is not open at that time, to the Emergency Room (ER) in NYULMC or Bellevue Hospital. The worker should also notify the CFAR Flow Cytometry Core Co-Directors and the Study Principal Investigator as soon as possible, after appropriate emergency care has been obtained.

Follow-up treatment for all exposures in the CFAR Flow Cytometry Core will be advised by, offered by or arranged by EHS or the ER. Form FLOW-102F BSL-2 Spill/Accident Report should be filed with the Laboratory Manager.

## 7. Standard Operating Procedures (SOPs) for the CFAR Flow Cytometry Core

- 7.1. A dedicated set of SOPs and forms are to be followed and used by all personnel using the CFAR Flow Cytometry Core.

The currently approved SOPs and forms pertaining specifically to the virus laboratory are on the following pages of this safety manual and are listed below:

Document No.	Name
SOP FLOW-101	Standard Laboratory Practices for the CFAR Flow Cytometry Core Laboratory
SOP FLOW-102	Spill Response and Reporting
SOP FLOW-103	Exposure Incidents and Reporting
SOP FLOW-104	Shipping and Receiving Infectious Substances and On-campus Transportation of Biological Samples
SOP FLOW-105	Medical and Facility Emergencies
Form FLOW-101F	BSL-2 Sorting User Approval
Form FLOW-102F	Core Facility Spill/Accident Report

<b>Standard Operating Procedures</b>
<b>Title:</b> Standard Laboratory Practices for the CFAR Flow Cytometry Core Laboratory
<b>SOP#:</b> FLOW-101
<b>Purpose:</b> To provide safe handling procedures and operations for all personnel working in the facility

**1. Materials**

Item	Manufacturer	Catalog No.
Lab Coats	-	NYU Building Services
Safety Glasses	Jackson Safety	19706-002
Gloves	Americare	NYU Requisition (Small 7-001) (Medium 7-002) (Large 7-003)
200 Proof Ethyl Alcohol	-	NYU Requisition
RelyOn Multi-purpose Disinfectant Wipes	DuPont Chemical	Fisher# 19-120-3881
Bleach	Clorox	Staples# CLO 02489
Kim Wipes	KimTech	34155
FACSFlow Sheath	BD	342003
Alcohol Swabs	BD	366894
Accudrop Beads	BD	345249
CST Beads	BD	641319
Buffalo ULPA Filters	Buffalo	BD# 335029
GloGerm Beads	GloGerm	-

**2. Restricted Access**

- 2.1. Entry into the CFAR Flow Cytometry Core Laboratory is restricted to authorized individuals who have received medical clearance from EHS, have taken the *Intro to Biosafety* training, the *OSHA Bloodborne Pathogens* self study, and reviewed the SOPs for the CFAR Flow Cytometry Core Laboratory.
- 2.2. NYULMC's Biosafety Officer/Specialist and Environmental Specialists will be granted access to conduct unannounced inspections.
- 2.3. Entry into the CFAR Flow Cytometry Core is restricted by a keyed lock and users must always be accompanied by a member of the Flow Core Staff unless otherwise authorized.
- 2.4. During sorting of potentially infectious agents, access to the laboratory will be restricted.

**3. General Facility Requirements**

- 3.1. Use of needles and other sharp instruments will not be used when biohazardous samples are present.
- 3.2. All cuts in the skin must be covered with a bandage.
- 3.3. No food or drinks are allowed.
- 3.4. No open-toed shoes are to be worn in the facility.
- 3.5. No jewelry (other than wedding bands) is to be worn under gloves.

- 3.6. No mouth pipetting is allowed in the facility.
- 3.7. All samples must be labeled with name, date and specimen type with a water/alcohol resistant marker.
- 3.8. Post-sort clean up should follow procedures listed in the *Decontamination and Exit out of the CFAR Flow Cytometry Core* sections of this SOP.

#### 4. Reagents and Supplies

- 4.1. All samples that are transported to the CFAR Flow Cytometry Core must be contained using approved secondary containers. Refer to SOP FLOW-104
- 4.2. Unopened, non-infectious, non-toxic reagents and supplies are stored in rooms CD643 or CD647.

#### 5. Entry to the CFAR Flow Core Laboratory and Personal Protective Equipment (PPE)

- 5.1. All outside clothing not worn under a lab coat must be left in room CD643 (office). Bags and anything not to be used in the Core Laboratory should be left there as well.
- 5.2. Wearing two pairs of gloves is required. They are disposed when overtly contaminated and removed when work is completed or integrity is compromised. Small, medium and large gloves are available to the left of the entrance to the room, and should be worn at all times. They will be sprayed with 70% ethanol or isopropyl alcohol as necessary and are not to be worn outside the lab.
- 5.3. Lab coats are available on a coat rack behind the door and are to be worn at all times while inside the lab. If a different size is needed, coveralls or surgical gowns can be supplied. Non-disposable lab coats are laundered on a regular basis by NYULMC Building Services.
- 5.4. The laboratory door should remain closed except when entering and exiting the lab.

#### 6. Aerosol generating procedures

All transfers of biohazardous materials from one container to another container must take place within a BSC. Such transfers may **not** take place on the open bench.

All other procedures that could generate aerosols must also be conducted in a BSC. The following are examples of these procedures:

- Mixing of samples with a pipette;
- Using high speed mixing devices like vortexers;
- Opening of centrifuge buckets; and
- Opening a package containing an infectious pathogen.

## 7. Use of FACSAria Flow Cytometer and Baker BioProtect III BSC

- 7.1. Any biohazardous or potentially biohazardous samples run on the FACSAria have the potential to aerosolize during instrument malfunction and contaminate the sorting chamber of the instrument. Because of this, sort setups must be preformed in a sequence. The setup procedure outlined below will insure that all necessary adjustments are made before any sample is run on the instrument.

Step	Procedure	Comments
1	Turn on the Baker BSC blowers and lights.	The blowers must be on for 15 minutes before any human samples are run.
2	Turn on the FACSAria instrument and computer.	The FACSAria needs at least 15 minutes to warm up (30 minutes for use of the UV laser) before step 6
3	Turn on Whisper Aerosol Management unit, and (optionally) the water recirculation unit.	Whisper Unit should be on for 15 minutes before any human samples are run.
4	Check sheath, waste and other solution levels.	Refill or empty as necessary at this time. Add bleach to waste tank as per <i>Decontamination</i> section of this SOP.
5	Run Fluidics Startup and turn stream on.	Let stream stabilize and set gap and drop. Engage "sweet spot."
6	Run CST beads for instrument QC.	
7	Run Accudrop beads and perform drop delay setup.*	
8	Set up sort side streams for appropriate collection vessels.*	
9	Change necessary optical filters.*	
10	Ensure hood hatches are closed, hood pressure gauge is correct and proper PPE is used.	
11	Begin running samples/sorting.	

\* Steps 7, 8, and 9 must be completed *before* a biohazardous sample is run on the machine. These steps require open hatches on the hood and can create aerosols.

- 7.2. In the event of a clog the stream may lose stability and generate an aerosol. In most cases, any aerosol should be contained within the sort block and filtered out by the Whisper Aerosol Management Unit. If the stream restarts correctly, the sort can continue.
- 7.2.1. If the sort block needs to be opened, or the collection containers removed after a clog, the operator must wait for 20 minutes before proceeding, to allow any aerosol to settle.
- 7.2.2. If the nozzle must be removed for cleaning, it should be treated as biohazardous. The stream should be turned off, and the nozzle taken out of the machine. Before removal from the hood it should be placed into a 15mL conical tube, filled with either diH<sub>2</sub>O or EtOH, which is then capped. This tube can then be taken out of the hood and placed into the sonicator for cleaning. The tube should not be opened again until inside the hood.

- 7.3. After sorting is complete the operator must wait for 5 minutes before removal of the collection apparatus. Tubes should be capped and plates covered. All collection containers should be labeled and wiped down with RelyOn Disinfectant Wipes before removal from the Biosafety cabinet.
- 7.4. To decontaminate the FACS Aria, a tube of 70% EtOH should be run on the sample port for 5 minutes at high speed (10). Afterwards, one of two built-in automated procedures must be used.
  - 7.4.1. If the machine is to be used again, the automated "Prepare for Aseptic Sort" procedure of the FACSDiva Software can be initiated. This procedure will flush all lines that come in contact with sample with 70% EtOH and disinfectant. Once complete the machine will be flushed with clean FACSFlow Sheath solution.
  - 7.4.2. If the machine is no longer needed for the day, the automated "Fluidics Shutdown" procedure of the FACSDiva Software can be initiated. This will also flush the machine with 70% EtOH and disinfectant, but will not flush the machine with Sheath solution. Using this procedure for shutdown will reduce salt buildup and help prevent future clogs.
- 7.5. The surfaces of the FACS Aria and Baker BioProtect Cabinet should be wiped down with 70% EtOH or RelyOn Disinfectant Wipes. Blowers should run for at least 15 minutes after cabinet has been wiped down.
- 7.6. Waste from the FACS Aria is pumped into a 10 liter tank on the fluidics cart below the hood. This tank must contain enough bleach (1 liter) to render the final volume in the tank 10% bleach. The tank is capped with a biohazard filter that allows air pressure release, but prevents aerosol from escaping. The filter is to be changed on a monthly basis.
8. **Use of Nuair Class II Type A BSC**
  - 8.1. Before working in the BSC, the blowers and fluorescent light are switched on, and a biohazard bag, a spray bottle of 70% isopropyl alcohol, wipers, and pre-saturated wipes are placed in the cabinet. The blowers must be left on for 15 minutes before use.
  - 8.2. All materials needed to complete the experiment are placed in the cabinet to limit the number of times hands pass through the air barrier. Equipment is not to be placed on the intake grills at the front of the cabinet, nor blocking the exhaust opening at the back of the cabinet.
  - 8.3. A biohazard bag should be present in the cabinet. Absorbent material (such as a dry cleanroom wiper) is placed in the bottom of the biohazard bag. This bag is used for discarding solid waste (gloves, plastic waste, pipette tips). Once the bag is full, it is closed, wiped with 70% isopropyl alcohol and taken out of the cabinet to be collected into a larger covered waste container next to the cabinet.
  - 8.4. Liquid waste should be put into a dedicated container inside the BSC with sufficient sodium hypochlorite to achieve a final concentration of 20-30% and allowed to react for a minimum of 1 hour before disposal. Wipe or spray the outside of the container with 70% ethanol or isopropyl alcohol before removing it from the cabinet. The decontaminated liquids are then disposed of down the sink and flushed with large amounts of tap water.
  - 8.5. Vacuum waste flasks should contain enough bleach to result in a 10% solution. They should never be filled more than 50%. An in-line vacuum filter must be present between the flask and the vacuum source.

- 8.6. Contaminated pipettes should be disposed of in the biohazard bags.
- 8.7. Anything removed from the BSC during the work session is to be decontaminated by wiping with 70% isopropyl alcohol while still in the BSC.
- 8.8. At the end of each work session, culture tubes, racks and other material to be removed from the cabinet are decontaminated by wiping with 70% isopropyl alcohol while still within the BSC.
- 8.9. The wipers used during cleaning along with the outer gloves are placed into a biohazard bag while still within the BSC. Wipe or spray the outside of the bag with 70% isopropyl alcohol. Place the bag into a larger covered biohazard waste container next to the cabinet.
- 8.10. A fresh pair of outer gloves is donned and the hood is now wiped down completely with 70% isopropyl alcohol.
- 8.11. All tissue or cell culture related materials should be disposable whenever possible. Only disposable plastic pipettes and plastic tubes are to be used in the facility.

## 9. CO<sub>2</sub> Incubators

The following is a list of safety practices and procedures for doing work involving the use of cell culture incubators.

**NOTE: The incubator in the CFAR Flow Cytometry Core is not available for tissue culture use. The incubator is to be used only to keep samples at 37 degrees C for short periods of time just prior to or preceding a sort.**

- 9.1. Flasks and culture plates shall be carried to and from the incubator using plastic secondary containers.
- 9.2. In the event of bacterial or fungal contamination in the incubators, all contents shall be moved to a BSC. Shelves shall be wiped down with 70% isopropyl alcohol and shelves should be sterilized in an autoclave.
- 9.3. Gloves must be worn when handling cultures.
- 9.4. Prior to maintenance, equipment must be decontaminated.

## 10. Centrifuge

The following is a list of safety practices and procedures for doing work involving the use of centrifuges.

- 10.1. Rotor buckets and lids shall be sprayed with 70% isopropyl alcohol and placed in the BSC prior to loading.
- 10.2. Samples shall be loaded into rotor/rotor buckets and sealed with the cap for the rotor bucket while in BSC.
- 10.3. After centrifuging, rotor/rotor buckets shall be moved to BSC to unload samples. Samples shall NOT be unloaded in the open room.
- 10.4. Centrifuge and rotor chambers shall be disinfected with 70% isopropyl alcohol soaked wipers

following use.

10.5. Prior to maintenance, equipment must be decontaminated.

## 11. Decontamination

Work surfaces are to be decontaminated on completion of work, after any spill or splash, or when switching over to a new patient or product batch.

Decontaminate as follows:

11.1. **Bench tops and external equipment surfaces:** Work surfaces are wiped down with 70% ethanol or RelyOn Disinfectant Wipes.

11.2. **Water baths and Sonicator:** Water baths and sonicators are completely emptied of water and wiped down with 70% ethanol or RelyOn Disinfectant Wipes.

11.3. **Biosafety cabinet work surfaces:** BSC work surfaces are sprayed with disinfectant cleaner (RelyOn Multi-purpose disinfectant cleaner or equivalent), allowing a 10 minute contact time, followed by wiping down with 70% isopropyl alcohol to remove excess disinfectant residue.

11.4. **Interior surfaces of equipment:** Interior surfaces of centrifuges (including centrifuge buckets), incubators and other large equipment are wiped down with 70% ethanol or RelyOn Disinfectant Wipes.

11.5. **Liquid Waste:** Liquid biohazard waste will be decontaminated with sufficient sodium hypochlorite to achieve a final concentration of 10% for a minimum of 1 hour and then emptied into the sink.

11.6. **Other Potentially Contaminated Waste:** All other potentially contaminated waste such as disposable lab coats and gloves are collected in red bags in containers with lids. Clothing that becomes contaminated with HIV preparations will be decontaminated by spraying with 70% ethanol before being laundered or discarded.

11.7. All red bags containing contaminated wastes must be double-bagged and securely sealed with tape. All sharps containers should be locked closed. Outside surfaces of both red bags and sharps containers must be wiped down with disinfectant cleaner (RelyOn Multi-purpose disinfectant cleaner or equivalent) before transporting out of the lab.

## 12. Use of Chemicals

12.1. The same practices and training requirements will apply to the use of chemicals as in all other laboratories of NYULMC. Specifically, personnel must be current with *Chemical Hygiene* and *Hazardous Waste* training requirements. EH&S offers training on the 2nd Thursday of each month; contact them @ x35159 for further information.

12.2. For all chemicals used in the facility, the user must give the Laboratory Manager a corresponding Material Safety Data Sheet (MSDS). All personnel must be instructed as to their importance and their location within the facility; the Laboratory Manager will be in charge of monitoring chemical storage and use within the facility.

## 13. Disposal of hazardous chemicals

13.1. Hazardous chemicals will be collected in properly labeled containers in a designated area in the

lab Arrangements for the disposal of hazardous chemical waste may be made by contacting EH&S.

13.2. Biohazard waste **cannot** be discarded through the Hazardous Waste Disposal Program.

13.3. Arrangements for the disposal of hazardous chemical waste that is also a biohazard may be made by contacting EH&S.

**14. Exit out of the CFAR Flow Cytometry Core**

14.1. All persons leaving the Core laboratory must remove PPE and wash hands before exiting.

14.2. Solid biohazard waste (red bags and sharps containers) should be stored in designated area, as there is no regular pickup and NYULMC Environmental Services must be notified for pickup.

14.3. Decontaminated liquid biohazard waste should be emptied into the sink and flushed with large amounts of tap water (*Refer to 11.5 in SOP#: FLOW-101 for proper liquid decontamination practices*).

14.3.1. Used liquid waste canisters should be disposed in red bag waste.

14.3.2. Secondary containers for carrying liquid waste containers are disinfected by spraying down with 70% ethanol or isopropyl alcohol, and may be autoclaved if needed.

14.4. Dispose gloves in a biohazard waste receptacle (red bag waste) and wash hands before exit.



## Standard Operating Procedures

Title: Spill Response and Reporting

SOP#: FLOW-102

Purpose: To provide safe procedures for spill response in the facility

### 1. Materials

Item	Manufacturer	Catalog No.
Biohazard Bags	Lab Guard	19075388E
RelyOn disinfectant cleaner	Dupont	Fisher # 19-120-3881
Blue absorbent pads	Fisherbrand	14-206-62
Spill Kit	Spill Defense	25916

### 2. Spill Response

2.1. Spills will be decontaminated promptly by the responsible party.

2.2. Personnel in the immediate area will be alerted and access to the contaminated area (around the spill) will be clearly marked with the biohazard floor sign and restricted.

### 3. Spill Clean-Up

3.1. Don a lab coat, two pairs of gloves, and eye or face protection

3.2. Use the spill kit to clean up the spill. The spill kit contains absorbent packets and pads.

3.3. Carefully cover the spill with the absorbent packets.

3.4. Taking care to avoid splashing pour a freshly prepared 1 in 10 dilution of bleach around the edges of the spill.

3.5. Allow a 30 minute contact time.

3.6. Pick up any glass with tongs

3.7. Use dry cleanroom wipers or the absorbent pads to wipe up the spill working from the edges into the center.

3.8. Disinfect the spill area by spraying thoroughly with RelyOn disinfectant/cleaner, allowing a 10 minute contact time before wiping dry.

3.9. Discard waste and any contaminated PPE in a red biohazard bag.

3.10. Wash hands.

### 4. Reporting

Spills or accidents will be reported to the Biosafety Officer/Specialist, the Core Laboratory Manager, and the Co-Directors of the CFAR Flow Cytometry Core. Fill out the *Core Facility Spill/Accident Report Form*, (Form **FLOW-102F** – a copy is at the end of this safety manual or can be obtained from the Laboratory Manager) to document large spills or other potentially serious accidents.

<b>Standard Operating Procedures</b>
<b>Title:</b> Exposure Incidents and Reporting
<b>SOP#:</b> FLOW-103
<b>Purpose:</b> To provide safe procedures for accidental exposures

**1. Emergency Procedures:**

All personnel who work in the lab will be familiar with the Emergency Response Guide for New York University Medical Center Laboratories that is posted in the lab next to the entrance. This gives basic information on responding to fire alarms, chemical or biological spills or personal injury.

**2. Exposure Incidents**

Manage exposure incidents such as cuts with contaminated instruments, or splash to mucous membranes as follows:

**2.1. For cuts with contaminated instruments:**

- 2.1.1. Stop work immediately.
- 2.1.2. Remove contaminated gloves and allow the wound to bleed freely for a minute under warm running water.
- 2.1.3. Wash the wound with soap and water for 15 minutes and apply sterile gauze or a bandage, if necessary.
- 2.1.4. Remove protective lab clothing and proceed immediately to the appropriate location for treatment and counseling.

**2.2. For splashes to mucosal membranes:**

- 2.2.1. Stop work immediately and proceed immediately to the eye wash station.
- 2.2.2. Rinse tissue surface with copious amounts of water. Eyes should be irrigated for at least 15 minutes.
- 2.2.3. Remove protective lab clothing and proceed immediately to the appropriate location for treatment and counseling.

**Appropriate Locations for Treatment and Counseling**

Department	Phone Number	Location	Hours of Operation
Employee Health Services	212-263-5020	660 First Avenue on the 2nd Floor	M-F 8:00AM-5:00PM
NYULMC Emergency Room	212-263-5550	560 First Avenue	Open 24 hours 7 days/week
Bellevue Hospital Emergency Department	212-562-5082	462 First Avenue	Open 24 hours 7 days/week

**Note:** If a laboratory worker has a parenteral (e.g. percutaneous injury or contact with non-intact skin) or mucous membrane exposure to blood, body fluid, or viral-culture material, the source material will be identified and, if possible, tested for the presence of virus. **In general, materials handled in**

**the CFAR Flow Cytometry Core should be considered contaminated unless known otherwise.**

For work involving HIV-infected or potentially infected materials, the worker must be escorted directly to the emergency room for immediate evaluation and counseling with regard to the risk of infection. EHS offers post-exposure prophylaxis (PEP) according to the most recent guidelines, and if deemed necessary, should begin as soon as possible, typically within hours of exposure. Administration of PEP should not be delayed for HIV test results.

As of August 2008, the CDC recommendation is as follows:

“Use of PEP with antiretroviral medications, initiated as soon as possible after exposure and continuing for 28 days, has been associated with a decreased risk for infection following percutaneous exposure in health-care settings (22)...Because of the potential toxicities of antiretroviral drugs, PEP is recommended unequivocally only for exposures to sources known to be HIV-infected. The decision to use PEP following unknown-source exposures is to be made on a case-by-case basis, considering the information available about the type of exposure, known risk characteristics of the source, and prevalence in the setting concerned.” [MMWR Aug 1, 2008 / 57(RR06); 1-19].

The worker will be evaluated serologically for HIV and advised to report and seek medical evaluation of any acute febrile illness that occurs within 12 weeks after the exposure. Such an illness – particularly one characterized by fever, rash, or lymphadenopathy – may indicate recent HIV infection. If the initial (at time of exposure) HIV test is negative, the worker should be retested 6 weeks after the exposure and periodically thereafter (i.e., at 12 weeks and 6, 9 and 12 months after exposure). During this follow-up period exposed workers should be counseled to follow Public Health Service recommendations for preventing transmission of HIV.

**NOTE: Please note that exposure to other bloodborne pathogens or other potentially infectious materials is discussed in detail in NYULMC’s *OSHA Bloodborne Pathogens* self study.**

### **3. Reporting**

Exposure incidents must be reported immediately either in person or by phone to a CFAR Flow Cytometry Core Manager, the Co-Directors of the CFAR Flow Cytometry Core, and EHS. Use *Core Facility Spill/Accident Report* Form, (Form **FLOW-102F** – a copy is at the end of this safety manual or can be obtained from the Laboratory Manager) to document the incident.

<b>Standard Operating Procedures</b>
<b>Title:</b> Shipping and Receiving Infectious Substances and On-campus Transportation of Biological Samples
<b>SOP#:</b> FLOW-104
<b>Purpose:</b> To ensure that shipping and receiving/transportation of specimens and cultures which harbor or are suspected of harboring pathogens is performed in a controlled and dedicated manner.

## 1. Training Requirements

Personnel who want to ship or receive infectious substances **must** be current with training requirements.

1.1. EH&S provides the self-study packages: *Introduction to Shipping Hazardous Materials* and *Shipping and Receiving Biological Materials*, which are available at:  
<http://redaf.med.nyu.edu/shipping-and-receiving-biological-materials-training>

1.2. A training certificate is issued and maintained in the EH&S Department upon successful completion of both post tests mentioned in 1.1; the certification is valid for **two** years.

## 2. On-campus Transportation of Biological Samples

### 2.1. Materials

Item	Manufacturer	Catalog No.
Biohazard Bags	Lab Guard	19075388E
Hard container (cooler)	Igloo	7362
Blue absorbent pads	Fisherbrand	14-206-62

### 2.2. General Notes

2.2.1. All samples and containers must have biohazard labels.

2.2.2. Avoid crowded areas whenever possible.

2.2.3. The container should be carried directly to the intended laboratory - do not take the container to offices, cafeterias or other public or inappropriate locations.

2.2.4. The package should be carefully inspected for signs of leakage or other contamination and, if necessary, decontaminated before opening.

### 2.3. Packaging Instructions

2.3.1. Label samples. Label information must include the identity of the biological material or agent, the universal biohazard symbol and the sending and receiving laboratory identification (*e.g., Principal Investigator name and room number*).

2.3.2. Place sample in a primary container which is sealed and leak proof.

2.3.3. Place the primary container in a secondary hard case container which is easy to decontaminate and capable of being securely closed.

2.3.4. Liquid samples should be surrounded by enough absorbent pads in the secondary container to contain any liquids and absorb any shock during transport.

## Standard Operating Procedures

Title: Medical and Facility Emergencies

SOP#: FLOW-105

Purpose: To provide safe procedures for handling medical and facility emergencies

### 1. Materials

Item	Manufacturer	Catalog No.
Emergency flashlight	Sexauer	FSI #456870
First Aid Kit	PhysiciansCare	Staples #503995
Smart Universal Power Supply 3000XL	ADP	-

### 2. Medical Emergencies

- 1.1. In case of a medical emergency, call the Medical Center's emergency number: 33-911.
- 1.2. If the individual is conscious and can be moved, remove him/her immediately out of the laboratories.
- 1.3. If the individual is unconscious and it will cause no further harm, the person will be immediately removed out of the laboratories and emergency personnel will be called to perform first aid.
- 1.4. If the victim cannot be moved, instruct the emergency responders of hazards and protective measures necessary in the facility.
- 1.5. Stay with the victim until emergency medical personnel arrive and take over.

### 2. Electrical Failures

- 2.1. In case of a power outage the operator must use his/her own best judgment to assess the situation and act accordingly.
- 2.2. In case of an electrical failure, call Bellevue's main number for Facilities: (212) 562-4779.
- 2.3. Loss of power to the essential containment equipment will be avoided by use of a APC Smart UPS 3000xL battery backup system that will maintain power to the Aria Flow Cytometer, Whisper Aerosol Containment Unit, BioSafety Cabinet, and Vacuum pump for up to two hours, allowing proper shutdown and containment of biohazards.
- 2.4. If the blower fan of a BSC stops working any operator working in the BSC is required to cease all work immediately and exit from the laboratory following the exit procedures listed in SOP FLOW-101 for removal of protective gear.
  - 2.4.1. The blower must be on for at least thirty minutes before work can resume.
- 2.5. In case of a blackout, all operators are to evacuate the facility. A rechargeable flashlight will be available for emergency use if needed.
- 2.6. Exit doors are identified with glow-in-the-dark exit signs which will allow the operator to find the exit door. Exit procedures listed in **SOP FLOW-101** will be followed.
- 2.7. A sign should be posted on the entrance door with a notice advising persons not to enter the

facility.

### 3. Fire Emergency

3.1. In the event of a fire the laboratory worker must take the following steps:

3.1.1. If the infectious material is stored as per lab requirements, the worker removes the PPE and exits the lab quickly as per exit procedures detailed in **SOP FLOW-101**, as required when he/she leaves.

3.1.2. If research involving the infectious material is in progress, the worker will determine if the agent can quickly be secured or whether it is quicker to destroy the material prior to leaving the lab as outlined in the section on **SOP FLOW-101 Decontamination – Liquid Waste**.

3.2. After evacuating the facility on account of fire, all workers will remain at a safe distance to offer directions to the facility and any information EH&S and/or Fire Department personnel may request. When they or Fire Department personnel arrive on the scene, all workers will follow their instructions.

## FORM FLOW-101F. BSL-2 Sorting User Approval

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

Date of birth: \_\_\_\_\_

Principal Investigator: \_\_\_\_\_ Department: \_\_\_\_\_

Title: \_\_\_\_\_ Work phone: \_\_\_\_\_

Home phone: \_\_\_\_\_ E-mail: \_\_\_\_\_

\_\_\_\_ I have completed, within the past year, the NYULMC training on bloodborne pathogens.

• \_\_\_\_ I have read (and received a copy of) the CFAR Flow Cytometry Core's SOPs and am familiar with:

- Safe working practices, which all persons in the facility are expected to follow
- Appropriate responses for spills in the laboratory, both within and outside of BSCs
- Decontamination procedures
- Procedures for medical, electrical and fire emergencies
- The Biosafety Microbiological and Biomedical Laboratories (BMBL) manual published and periodically updated by the Center for Diseases Control (CDC), specifically section IV – Laboratory Biosafety Level Criteria, and section VIII E – Viral Agents (HIV/SIV).

\_\_\_\_ In compliance with the OSHA Bloodborne Pathogens Standard, NYULMC has an HBV vaccination program. I understand that under this program, any worker who is at risk from HBV from occupational exposure to human blood, blood products or body fluids, or HBV contaminated materials may receive an HBV vaccination free of charge. The HBV vaccination program is administered by EHS.

\_\_\_\_ I understand that the EHS and the ER are prepared to administer medications to reduce the risk of HIV infection following a needlestick or mucous membrane exposure to HIV and it is my responsibility to report immediately to be evaluated for such treatment in the event of a possible exposure.

User signature: \_\_\_\_\_ Date: \_\_\_\_\_

CFAR Core Laboratory signature: \_\_\_\_\_ Date: \_\_\_\_\_

**Form FLOW - 102F. Core Facility Spill/Accident Report**

**Reporting Objective:**

In the process of investigating and reporting incidents the facility can determine the cause and provide recommendations for future prevention and correction of the events that lead to the accident/spill. This document is based on OSHA CLP 02-00-135-Recordkeeping Policies and Procedures Manual (effective-12/30/2004).

If additional space is needed to complete any question for a section, please attach extra page indicating which section is being continued.

1. Completed by (Name, Job Title):

\_\_\_\_\_

2. Name/Job Title/Name of Principal Investigator: \_\_\_\_\_

3. Date/Time of Incident: \_\_\_\_\_

4. Infectious agent/hazardous substance involved: \_\_\_\_\_

5. Where did incident happen (which area of the Core facility)? \_\_\_\_\_

6. Describe circumstances that lead to incident (work being done at that time, location of spill, equipment involved):

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

7. Other persons in Core lab at time of incident (where were they; did they contribute to the incident?)

\_\_\_\_\_

8. Duration of safety breach (time to containment): \_\_\_\_\_

9. What, if any, measures were taken to contain the safety problem?

a. Evacuation of facility \_\_\_ Yes \_\_\_ No

b. Who de-contaminated the spill (person or persons)? \_\_\_\_\_

\_\_\_\_\_

10. Who was notified of the incident? When were they notified?

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

11. List any injuries as a result of this incident:

\_\_\_\_\_  
\_\_\_\_\_



12. What medical evaluation or treatment was sought due to the incident?

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- a. Emergency Room \_\_\_\_NYULMC \_\_\_\_Bellevue HC \_\_\_\_No
- b. Employee Health Services \_\_\_\_Yes \_\_\_\_No
- c. If any other medical/healthcare treatment was obtained outside the work-site, where was it obtained:

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13. Please suggest any future measures that could be taken to prevent a recurrence of this type of incident:

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