SAFETY MANUAL
AND
STANDARD OPERATING PROCEDURES

FOR THE

DART Cytometry and Cell Sorting Laboratory

NEW YORK UNIVERSITY
SCHOOL OF MEDICINE

Locations

Skirball Institute, Laboratory 3-9 back
Old Bellevue C&D building, Room 645
Alexandria Center for Life Sciences, West Tower, Rm 329
New Science Building, Rm 449

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List of Key Personnel

Name	Title	Extension	Mobile
Peter Lopez	Director	x30635	646-357-0168
Michael Gregory	Technical Director	x35907	516-641-5185
Kamilah Ryan	Asst Research Scientist	x35907	917-715-5337
Yulia Chupalova	Asst Research Scientist	x35907	203-570-3649
Colin Zollo	Asst Research Scientist	x35907	203-430-8189
Barbara Muesing	Laboratory Technician	x35907	917-664-8492

Important Telephone Numbers

Note: *In an emergency, Communications can page personnel from key departments*	
Any Medical Center Emergency	x33911
NYULH Building Services	x35071
NYULH Communications	x37403
NYULH Emergency Department (ED)	x35550
Bellevue Emergency Department (ED)	(212) 572-5082
NYULH Occupational Health Services	x35020
Environmental Health and Safety (EH&S) Mark Olmsted, Environmental Specialist II Paul Rubock, Institutional Biosafety Officer Sayra St. Omer, Environmental Specialist II	x35159 x35161 x16774 x10593
NYULH Facilities Management	x35275
Bellevue Facilities Management	(212) 562-4779
Alexandria Center for Life Sciences, NYULH Contacts Brandan Gillespie, Tenant Coordinator John Wefels, Tenant Coordinator Tom Karagiannis, Facilities Manager Artemis (Tammy) Konstantinidis	 (646)-574-1421 (917)-242-0105 (646)-754-7371 (646)-532-9690 (cell) 646-574-7189
Alexandria Center for Life Sciences, East Tower Security Desk	646-439-1600
Alexandria Center for Life Sciences, West Tower Security Desk	646-440-9200
Poison Control	(212) 764-7667
NYULH Radiation Safety	x36888
NYULH Security	x73000
NYULH Environmental Services	x34930
Institutional Biosafety Committee (IBC) Taina LoSasso, Project Coordinator	(646) 754-5258 (646) 754-4640

1. Background

1.1. General Information

The DART Cytometry and Cell Sorting Laboratory consists of multiple locations in order to better serve the NYULH research community. At several of these sites biohazardous materials are processed. These include Skirball Floor 3, Laboratory 9; Bellevue CD645; and ACLS West 329 and 430. Instrumentation in these facilities have the potential to generate aerosols, thus special equipment and procedures are employed in these areas to contain potential biohazardous aerosols and reduce chance for operator exposure. For more detailed information please see the ISAC web site: http://isac-net.org/Resources/Biosafety.aspx .

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 additional precautions. 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, any recombinant agents requiring BSL2 containment as per the IBC, 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.

2.3. Flow Cytometric Cell Analysis

Unlike cell sorting, cell analysis involves the reading of cellular samples without physically separating them, thus, no aerosols are generated. Because of this, secondary containment is not essential but special precautions must still be taken when handling potentially biohazardous material. BSL2 safety guidelines involving safe handling of biohazardous specimens and proper PPE still apply and must be followed.

3. Containment

3.1. Biosafety Cabinets (BSCs) and Aerosol Management Units

3.1.1. High-speed cell sorters are contained in (at least) Biosafety Class I cabinets to contain potential aerosols generated as part of either the regular operation of cell sorters or during malfunction of cell sorters. Additional BSCs are used for cell culture and flow cytometry sample preparation.

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

3.1.3. Aerosol Management – Several of the cell sorters contained in BSCs also use a separate Aerosol Management System to remove and filter aerosols from the cytometer's interior compartments. Some units are integrated into the cabinet and uses the cabinet's filters while others are entirely separate units. These units can be used to quickly purge the sort chamber in the event of an instrument clog.

3.2. 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. Restricted Access

4.1.1. Entry into the DART Cytometry and Cell Sorting Laboratory facilities is restricted to authorized individuals who have received medical clearance from Occupational Health Services, have taken the *Intro to Biosafety* training, the annual *OSHA Bloodborne Pathogens* training available on FOCUS or in-person, and reviewed the SOPs for the DART Cytometry Laboratory.

4.1.2. NYULH's EH&S department will be granted access to conduct unannounced inspections.

4.1.3. Entry into the DART Cytometry and Cell Sorting Laboratory facilities is controlled by key, code or ID card-locked doors and users must always be accompanied by a member of the Laboratory Staff unless otherwise authorized.

4.2. Refer to **CCSL Location** documents below for location specific procedures.

5. General Facility Requirements

5.1. Needles and other sharp instruments will not be used when biohazardous samples are present.

5.2. All cuts in the skin must be covered with a bandage.

5.3. No food or drinks are allowed.

5.4. No open-toed shoes are to be worn in the facility.

5.5. No jewelry (other than wedding bands) is to be worn under gloves.

5.6. No mouth pipetting is allowed in the facility.

5.7. All samples must be labeled with name, date and specimen type with a water/alcohol resistant marker.

5.8. Follow procedures listed in the **CCSL SOP_112**, **Decontamination and Chemical Use** and the *Entry* and *Exit out of the DART Cytometry and Cell Sorting Laboratory* sections of the appropriate **CCSL Location** document.

6. General Materials

Item	Manufacturer	Catalog No.
Lab Coats	-	NYULH Building Services
Safety Glasses	Jackson Safety	19706-002
Gloves	Evolution One	Small EV-2050-S Medium EV-2050-M Large EV-2050-L
200 Proof Ethyl Alcohol	Decon Labs	2701
Bleach	Clorox	Staples# CLO 02489
Kim Wipes	KimTech	34155

7. Reagents and Supplies

7.1. All samples that are transported to the DART Cytometry and Cell Sorting Laboratory sites must be contained using approved secondary containers. Refer to **CCSL SOP_113**.

7.2. Unopened, non-infectious, non-toxic reagents and supplies may be stored in neighboring rooms or spaces.

8. Training

The DART Cytometry and Cell Sorting Laboratory is a Shared Resource Laboratory and as such has many users. Prior to being allowed independent access to or performing work independently in the facility, all personnel will be trained by an approved staff member and must be approved by the Director of the DART Cytometry Laboratory. Users accessing the laboratory for operated assisted work will always be accompanied by a staff member.

Training will include knowledge of the Safety Manual and approved protocols, followed by observation of a certified user performing the intended procedures. Training is requested using the iLab LIMS software. At this time the user acknowledges familiarity with these SOPs. Then the trainee will work under supervision of a certified lab staff until the certified user gives approval and has successfully completed all training requirements, the new user is certified to enter the laboratory, book instrumentation or work independently in the facility.

9. Medical Requirements, Surveillance, and Responses to Exposure

9.1. Medical Requirements

Workers with a known immunodeficiency disease or who are taking immunosuppressive medications are not permitted to work in the sorting room without prior approval by the DART Cytometry Director, EH&S, and Occupational Health Services. Workers with open wounds that cannot be adequately covered cannot work in the sorting room. Occupational Health Services can provide medical advice to workers who are not sure whether they fall into any of these exclusionary categories.

9.2. Medical Precautions and Surveillance

All NYULH personnel working with patient samples are offered a Hepatitis B (HBV) vaccination at their Employee Health Screening. Personnel working with bloodborne pathogens or other potentially infectious materials can obtain an HBV immunization by contacting Occupational Health Services at 212-263-5020. Occupational Health Services can also test whether an HBV immunization is still effective.

Non-NYULH employees are responsible for maintenance of their HBV immunizations and for their own HIV testing in the event of a Bloodborne Pathogens exposure.

9.3. 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 NYULH employees and as such apply to all employees working in the DART Cytometry Laboratory. These procedures are stated below.

All cuts and other exposures to blood or other body fluids must be reported *immediately* to Occupational Health Services, or, if not open at that time, to the Emergency Department (ED)

in the Tisch building. The worker should also notify the DART Cytometry Director and the Study Principal Investigator as soon as possible, after appropriate emergency care has been obtained.

Follow-up treatment for all exposures in the DART Cytometry Laboratory will be advised by, offered by or arranged by Occupational Health Services or the ER. **Facility Accident Report Form**, (Form **CCSL Form_F101** – a copy is at the end of this safety manual or can be obtained from the Laboratory Manager or Director) should be filed with the Laboratory Manager or Director. In addition, complete and injury report or EOIIR form which will be provided by the Emergency Department or Occupational Health Services. Exposure incidents involving rDNA must be reported to the IBC after the above steps have been taken.

10. Location Specific Requirements and Procedures

10.1. Requirements and procedures specific to individual locations are on the following pages of this safety manual and are listed below:

Document No.	Location
CCSL Location_1	Skirball Institute, Lab 3-9
CCSL Location_2	Bellevue Hospital, C&D building, Room 645
CCSL Location_3	ACLS West 329
CCSL Location_4	ACLS West 430

11. Standard Operating Procedures (SOPs) for the DART Cytometry and Cell Sorting Laboratory

11.1. A dedicated set of SOPs and forms are to be followed and used by all personnel using the DART Cytometry Laboratory.

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

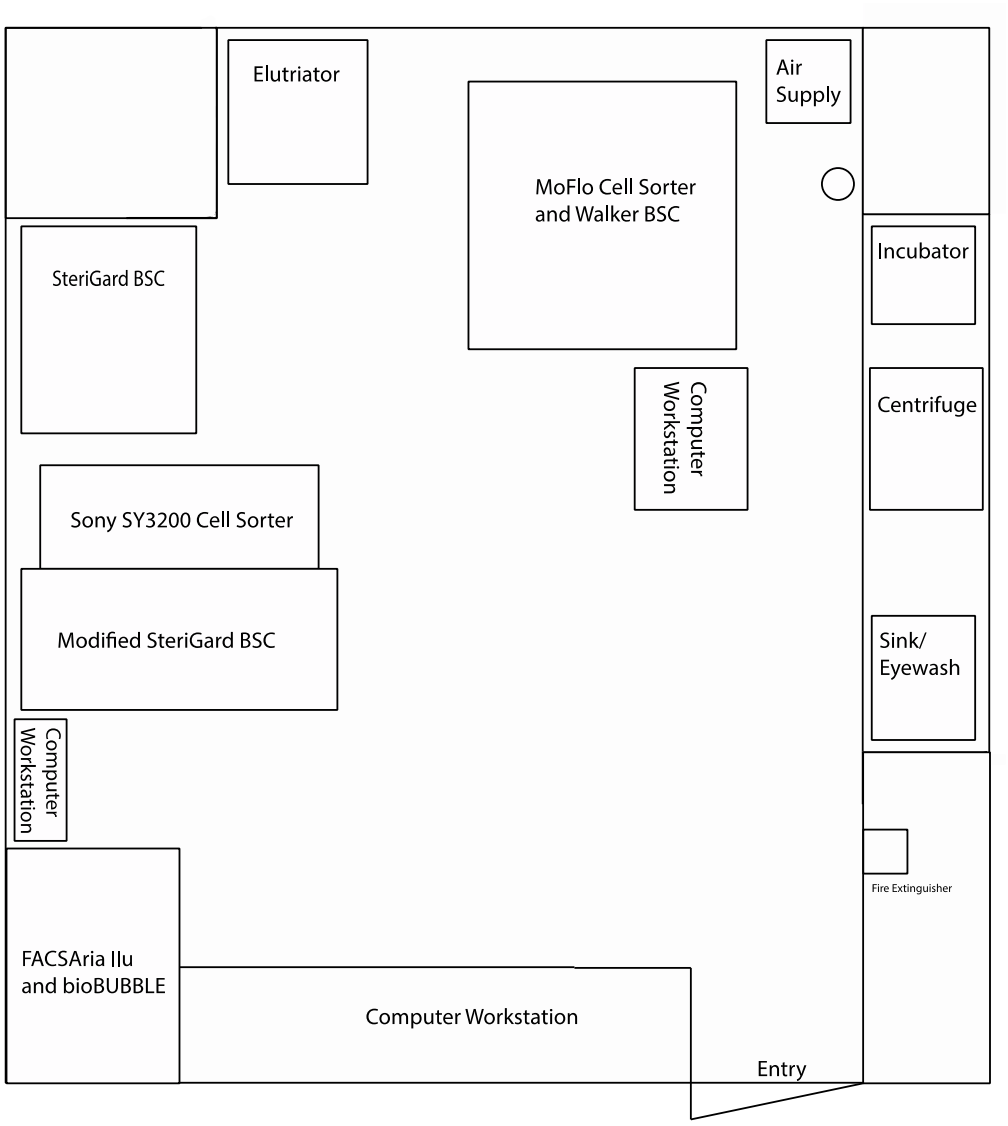
Document No.	Name
CCSL SOP-101	Moflo XDP Skirball Cell Sorter
CCSL SOP-102	SY3200 Skirball Cell Sorter
CCSL SOP-103	FACSAria IIu Skirball Cell Sorter
CCSL SOP-104	FACSAria IIu Bellevue Cell Sorter
CCSL SOP-105	Cytoflex ACLS West 329
CCSL SOP-106	ZE5 ACLS West 329 and 430
CCSL SOP-107	SH800 ACLS West 430
CCSL SOP-108	Aerosol Containment Testing
CCSL SOP-109	Tissue Culture BSCs

CCSL SOP-110	Centrifuges
CCSL SOP-111	CO2 Incubators
CCSL SOP-112	Decontamination and Chemical Use
CCSL SOP-113	Shipping and Receiving
CCSL SOP-114	Medical and Facilities Emergencies
CCSL SOP-115	Exposure Incidents
CCSL SOP-116	Spill Response
CCSL Form-F101	Facility Accident Report

Location Document
Title: Skirball 3-9 (Rear) Laboratory Layout
Location#: CCSL Location_1
Purpose: To provide safe handling procedures and operations for all personnel working in this facility.

The Skirball DART Cytometry and Cell Sorting Laboratory is a Biosafety Level 2 (BSL-2) certified facility that is located on the 3rd floor of the Skirball Institute, in the back room of laboratory 3-9. The facility includes a front room, used to store laboratory supplies and house instruments not requiring containment. The main laboratory room contains three Class II re-circulating biosafety cabinets, two housing cell sorting instruments and one for sample preparation/tissue culture. There is also a negative pressure enclosure around an additional cell sorter.

1.1. Skirball 3-9 Laboratory Facilities



1.1.1. The laboratory has one sink for hand washing near the entrance to the back room of Skirball 3-9. An eye wash station is located near the sink. An emergency shower is at main the entrance to Lab 3-9. The eyewash within the lab can also serve the function of the emergency shower.

1.1.2. The eye wash station and emergency shower are maintained and inspected periodically by the NYULH Facilities Department (212-263-5275). Weekly inspection and flushing of the eyewash is completed by the Cytometry Lab staff and issues are reported to the Facilities Department.

1.1.3. The facility is cleaned and maintained by the laboratory staff supplemented by Collins Building Services (CBS), a contractor managed by Environmental Services.

1.2. Restricted Access

1.2.1. A locking door to the rear and middle rooms are controlled by key or NYULH ID card and restricts access to this facility. Entry into the DART Cytometry Laboratory is restricted to authorized individuals who have received medical clearance from Occupational Health Services, have taken the *Intro to Biosafety* training, the *OSHA Bloodborne Pathogens* training on FOCUS and in-person, and reviewed the SOPs for the DART Cytometry Laboratory.

1.2.2. NYULH's EH&S department will be granted access to conduct unannounced inspections.

1.2.3. Untrained users must always be accompanied by a member of the Cytometry Staff unless otherwise authorized.

1.2.4. During sorting of potentially infectious agents, access to the laboratory will be restricted.

1.3. Biosafety Cabinets (BSCs), Enclosures, and Aerosol Management Unit

1.3.1. There is one three-foot Baker SterilGardIII Advance Class II BSC (not used for cell sorting), one modified Baker SterilGard Class II BSC, one Walker Class II BSC, and one bioBUBBLE Class I enclosure located in the facility. The Baker Sterilgard III Advance Class II BSC is used for cell culture, centrifuge elutriation sample preparation, and flow cytometry sample preparation. The modified Baker SterilGard cabinet houses the Sony SY3200 cell sorter and provides extra bench space for sample preparation. The Walker Class II BSC houses the Beckman Coulter MoFlo XDP cell sorter. The bioBUBBLE houses the FACSAria IIu cell sorter. See **CCSL SOP_109**, **CCSL SOP_102**, **CCSL SOP_101**, and **CCSL SOP_103** for guidelines on use of these biosafety cabinets and equipment.

Name	Room	Model	Serial No.
Baker SterilGard III Advance	SK 3-9	SG303	91620
Baker Modified SterilGard	SK 3-9	SG403A-HE-M	107613
Walker Medical Safety Cabinet	SK 3-9	Class II MSC Walker	WSC 1751A
Propel Labs bioBUBBLE	SK 3-9	bioBUBBLE	-

1.3.1.1. The modified Baker SterilGard cabinet includes a separate Aerosol Management System (AVAC) to remove and filter aerosols from the cytometer's interior compartments. The unit is integrated into the cabinet and uses the cabinet's filters

but operates from a separate blower. This unit is used to quickly purge the sort chamber in the event of an instrument clog.

1.3.1.2. The bioBUBBLE includes a tubing connection that draws aerosols directly from the sorting compartments of the cell sorter directly to the HEPA filter.

1.3.1.3. The Walker Medical Safety cabinet includes no additional aerosol management solution.

1.4. Additional equipment

1.4.1. Flow Cytometers and Cell Sorters

Name	Room	Serial No.	SOP #
Molfo XDP Cell Sorter	SK 3-9	5693614	**CCSL SOP_101**
SY3200 Cell Sorter	SK 3-9	S04-0017B	**CCSL SOP_102**
FACSAria II Cell Sorter	SK 3-9	P46900017	**CCSL SOP_103**

1.4.2. Incubators

1.4.2.1. There is one ThermoElectron Forma Series II Incubator located in the facility. This incubator is used to culture human and non-human cells. Proper guidelines for use of this incubator can be found in **CCSL SOP_111**.

Name	Room	Model	Serial No.
Forma Series II Incubator	SK 3-9	HEPA Class 100	307622-29942

1.4.3. Centrifuges

1.4.3.1. There is one Beckman Coulter Allegra X-12R centrifuge and one Beckman Coulter Avanti microcentrifuge located in the facility. These devices are used during sample preparation and tissue culture procedures. Proper guidelines for use of these centrifugal devices can be found in **CCSL SOP_110**.

Name	Room	Model	Serial No.
Beckman Coulter Allegra X-12R Centrifuge	SK 3-9	Allegra	504525
Beckman Coulter Avanti Microcentrifuge	SK 3-9	J-20XPI	6236312

1.5. Entry to the DART Laboratory and Personal Protective Equipment (PPE)

1.5.1. All outside clothing not worn under a lab coat must be left in the middle room of SK 3-9. Bags and other personal effects not to be used in the Laboratory should be left in SK 3-9 middle room as well.

1.5.2. Wearing two pairs of gloves is advisable. They are disposed when overtly contaminated and removed when work is completed or integrity is compromised. Small, medium and

large (latex or nitrile) gloves are available to the right of the entrance to the room, and should be worn at all times. Gloves are not to be worn outside the lab.

1.5.3. Lab coats are available on a coat rack next to entrance of the back room of SK 3-9 BSL-2 laboratory 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 NYULH Building Services.

1.5.4. The laboratory door should remain closed except when entering and exiting the lab.

1.6. Aerosol generating procedures

1.6.1. 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.

1.6.2. 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;
- Opening a package containing an infectious pathogen; and,
- Operation of the SY3200, MoFlo or FACSAria II Cell Sorters (SOP# specified above).

1.7. Exit from the DART Cytometry Laboratory

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

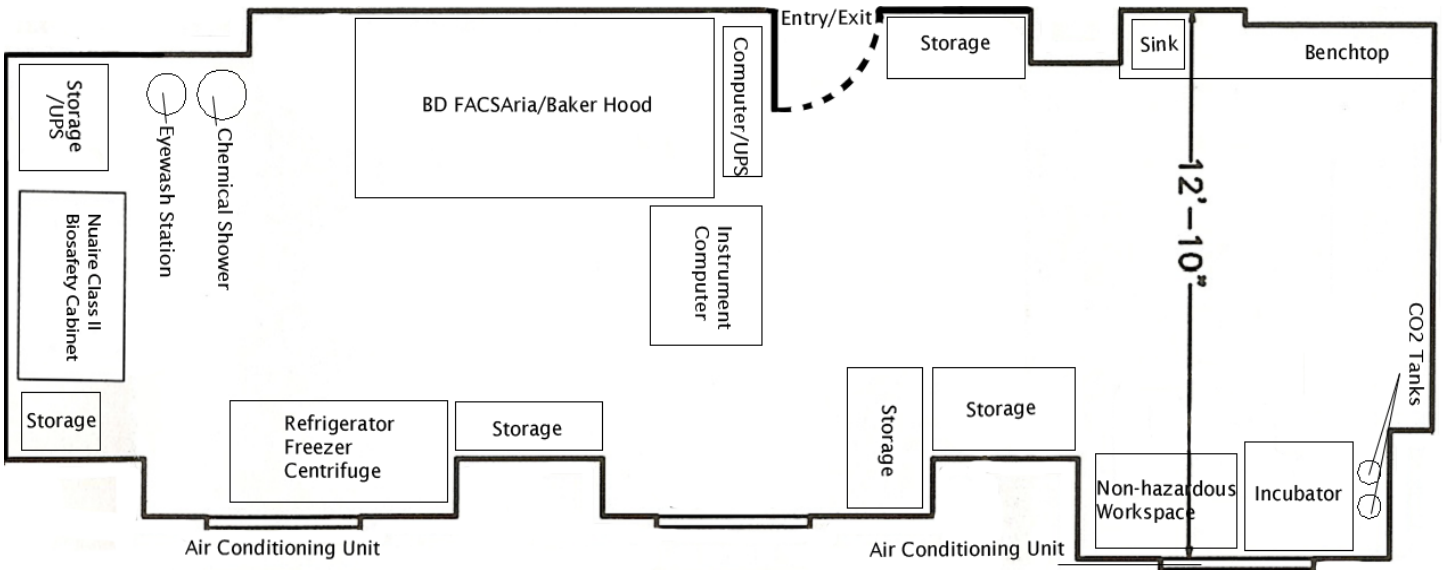
1.7.2. Decontaminated liquid biohazard waste should be emptied into the sink and flushed with large amounts of tap water (Refer to 1.5 in **CCSL SOP_112** for proper liquid decontamination practices).

1.7.3. Dispose of gloves in a biohazard waste receptacle (red bag waste) and wash hands before exiting.

Location Document
Title: Bellevue CD645 Laboratory Layout
Location#: CCSL Location_2
Purpose: To provide safe handling procedures and operations for all personnel working in this facility.

The Bellevue CD645 DART Cytometry and Cell Sorting Laboratory is a Biosafety Level 2 (BSL 2) certified facility that is located on the 6th floor of the OB-CD Building of Bellevue Hospital. The facility also includes an analysis room and an office, used to store laboratory supplies and user/staff possessions, rooms CD647 and CD643, respectively. 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 Cytometry & Cell Sorting Laboratory should be left there as well. The main laboratory room contains two Class II re-circulating biosafety cabinets, one housing the cell sorting instrument and one for sample preparation/tissue culture.

2.1. Bellevue CD645 Laboratory Facilities



2.1.1. The laboratory has one sink for hand washing near the entrance CD645. An eye wash station is located on the south end of the room, between the two biosafety cabinets. An emergency shower located next to the eye wash station and a second is located down the hall from the entrance to CD645.

2.1.2. The eye wash station and emergency shower are maintained and inspected by the Bellevue Hospital Facilities Department (212-562-5106). Weekly inspection and flushing of the eyewash is completed by the Cytometry Lab staff and issues are reported to the Facilities Department.

2.1.3. The facility is cleaned and maintained by the laboratory staff supplemented by Collins Building Services (CBS) a contractor managed by Environmental Services.

2.2. Restricted Access

2.2.1. A locking door to CD645 is controlled by key code and restricts access to this facility. Entry into the DART Cytometry Laboratory at Bellevue 645 is restricted to authorized individuals who have received medical clearance from Occupational Health Services,

have taken the *Intro to Biosafety* training, the *OSHA Bloodborne Pathogens* training on FOCUS and in-person, and reviewed the SOPs for the DART Cytometry & Cell Sorting Laboratory.

2.2.2. NYULH's EH&S department will be granted access to conduct unannounced inspections.

2.2.3. Untrained users must always be accompanied by a member of the Cytometry Lab Staff unless otherwise authorized.

2.2.4. During sorting of potentially infectious agents, access to the laboratory will be restricted.

2.3. Biosafety Cabinets (BSCs) and Aerosol Management Unit

2.3.1. There is one four foot Nuaire 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	Model	Serial No.
Baker BioProtect III BSC	CD645	BioProtect III	102073
Nuaire BSC	CD645	Nu-425-400	14398 ST

2.3.1.1. 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 tested by TSS and changed at their direction or if airflow indicator is below 3 inches WC. If the filter is changed proper PPE (lab coat and gloves) must be worn and the used filter is disposed of as biohazard waste. There is a second Whisper unit in the lab which can be used as a spare.

2.4. Additional equipment

2.4.1. Flow Cytometers and cell sorters

Name	Room	Serial No.	SOP #
FACSAria IIu Cell Sorter	CD645	P46900067	**CCSL SOP_104**

2.4.2. Incubators

2.4.2.1. There is one NAPCO Incubator located in the facility. This incubator only used to store human and non-human cells prior or post cell sorting. Proper guidelines for use of this incubator can be found in **CCSL SOP_111**.

Name	Room	Model	Serial No.
NAPCO Incubator	CD645	CO2 6000	602071969

2.4.3. Centrifuges

2.4.3.1. There is one Sorvall centrifuge and one Eppendorf microcentrifuge located in the facility. These devices are used to during sample preparation and tissue culture

procedures. Proper guidelines for use of these centrifugal devices can be found in **CCSL SOP_110**.

Name	Room	Model	Serial No.
Sorvall Centrifuge	CD645	RT7 PLUS	None
Eppendorf Microcentrifuge	CD645	5417R	5407 14382

2.5. Entry to the DART Cytometry Laboratory and Personal Protective Equipment (PPE)

2.5.1. 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 Cytometry & Cell Sorting Laboratory should be left there as well.

2.5.2. 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. Gloves are not to be worn outside the lab.

2.5.3. 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 NYU Langone Health Building Services.

2.5.4. The laboratory door should remain closed except when entering and exiting the lab.

2.6. Aerosol generating procedures

2.6.1. 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.

2.6.2. 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;
- Opening a package containing an infectious pathogen; and,
- Operation of the FACSAria IIu Cell Sorter (SOP# specified above).

2.7. Exit out of the DART Cytometry & Cell Sorting Laboratory

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

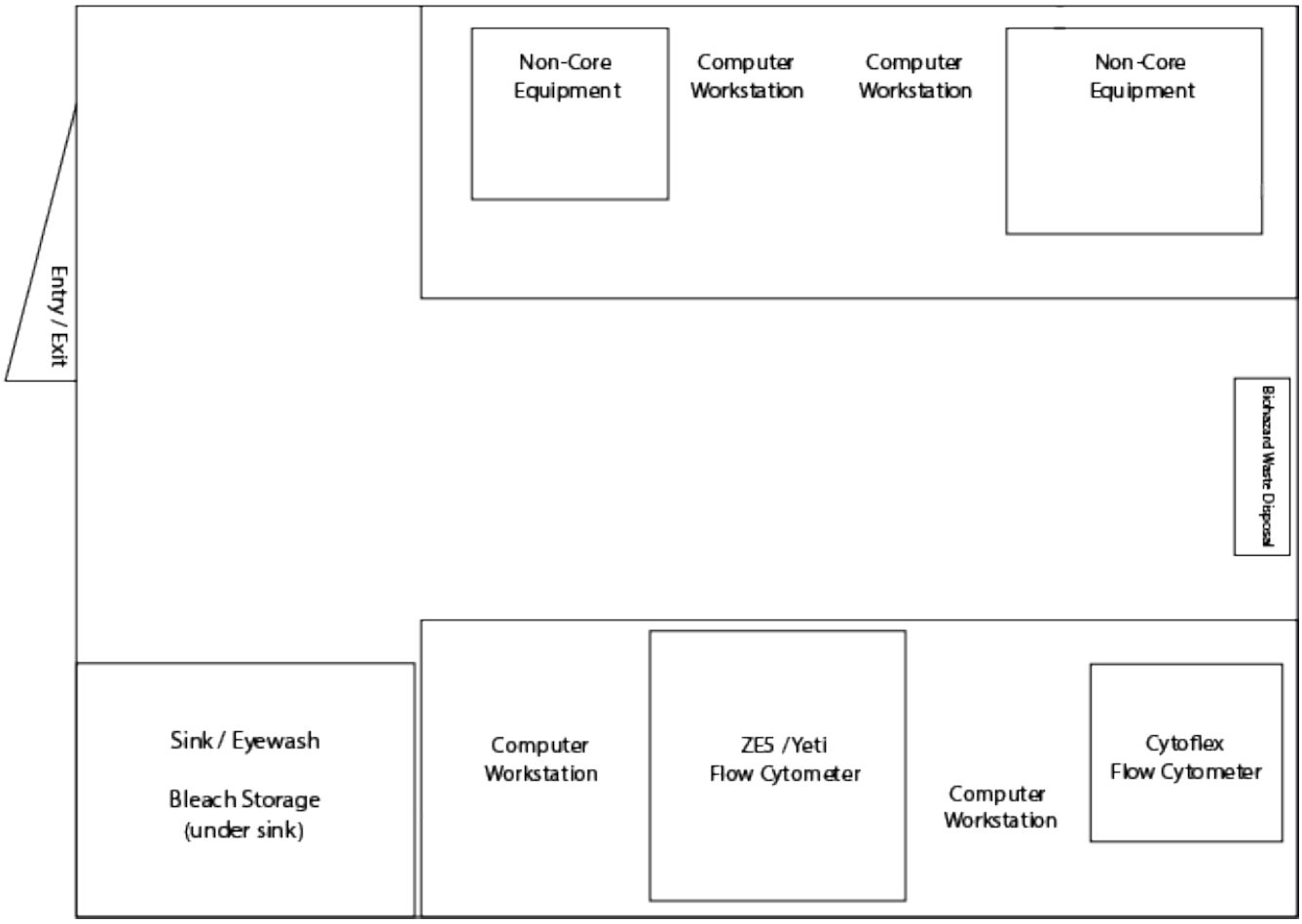
2.7.2. Solid biohazard waste (red bags and sharps containers) should be stored in designated area, as there is no regular pickup and NYU Langone Health Environmental Services must be notified for pickup.

1.7.4. Decontaminated liquid biohazard waste should be emptied into the sink and flushed with large amounts of tap water (Refer to 1.5 in **CCSL SOP_112** for proper liquid decontamination practices).

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

The DART Cytometry & Cell Sorting Laboratory is a Biosafety Level 2 (BSL 2) certified facility that is located in the Alexandria Center for Life Science's West Tower in room 329. This is a shared space housing equipment from both the Cytometry and Cell Sorting Laboratory as well as the Torres Laboratory. The Torres Laboratory equipment in the room are not covered by the Cytometry Lab SOPs, they are covered by the Torres lab SOPs. The instruments in this facility do not pose a risk for creating aerosols. The Cytometry Lab equipment in the room include a ZE5 Flow Cytometer and a CytoFlex Flow Cytometer.

3.1. Laboratory Facilities



3.1.1. The laboratory has one sink for hand washing directly to the right of the entrance. An eye wash station is located at the sink. An emergency shower is located just outside the room to the right. An additional shower is located at the breakroom entrance to the western lab area.

3.1.2. The eye wash station and emergency shower are maintained and inspected by Alexandria Center contractor, Penguin. Weekly inspection and flushing of the eyewash is completed by the Cytometry Lab staff and issues are reported to ACLS and Penguin.

3.1.3. The facility is cleaned and maintained by Collins Building Services (CBS), supplemented by the laboratory staff.

3.2. Restricted Access

3.2.1. Entry into the DART Cytometry & Cell Sorting Laboratory is restricted to authorized individuals who have received medical clearance from OHS, have taken the *Intro to Biosafety* training, the *OSHA Bloodborne Pathogens* self-study, and reviewed the SOPs for the DART Cytometry & Cell Sorting Laboratory.

3.2.2. NYULH's Biosafety Officer/Specialist and Environmental Specialists will be granted access to conduct unannounced inspections.

3.2.3. Entry into the DART Cytometry & Cell Sorting Laboratory is restricted after hours and on weekends to NYULH ID cardholders only.

3.2.4. The space is shared with the Torres Laboratory and as such, users of their equipment have physical access to the space. However, the Cytometry Lab instrumentation cannot be operated without software account creation, done after Cytometry Lab training is complete – this restricts use of the equipment.

3.3. Additional Equipment

3.3.1. Flow Cytometers and Cell Sorters

Name	Room	Serial No.	SOP #
Cytoflex Flow Cytometer	ACLS W 329	25831494	**CCSL SOP_105**
ZE5 Flow Cytometer	ACLS W 329	17	**CCSL SOP_106**

3.4. Entry to the DART Laboratory and Personal Protective Equipment (PPE)

3.4.1. All outside clothing not worn under a lab coat such as coats, hats, and so forth, are not permitted and should be secured outside of the facility before entering. Bags and anything not to be used in the Core Laboratory are subject to this as well.

3.4.2. It is the user's responsibility to have gloves with them when they enter the facility. The Core does not provide this resource to its satellite locations. Wearing two pairs of gloves is required. They are disposed of when overtly contaminated and removed when work is completed or integrity is compromised. Gloves are not to be worn outside the lab.

3.4.3. Lab coats are the user's responsibility and are to be worn at all times while inside the lab. Non-disposable lab coats are laundered on a regular basis by NYULH Building Services.

3.4.4. The laboratory door should remain closed except when entering and exiting the lab.

3.5. Aerosol generating procedures

3.5.1. 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. As the Cytoflex and ZE5 analyzers are not located in a biosafety cabinet, users conducting cell
3.5.2. analysis must have prepared their samples completely ahead of time.

3.5.3. 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;
- Opening a package containing an infectious pathogen; and,
- Operation of the ZE5 or Cytoflex analyzers (SOP#s specified above).

3.6. Exit out of the DART Cytometry and Cell Sorting Laboratory

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

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

3.6.3. Decontaminated liquid biohazard waste should be emptied into the sink and flushed with large amounts of tap water (Refer to 1.5 in **CCSL SOP_112** for proper liquid decontamination practices).

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

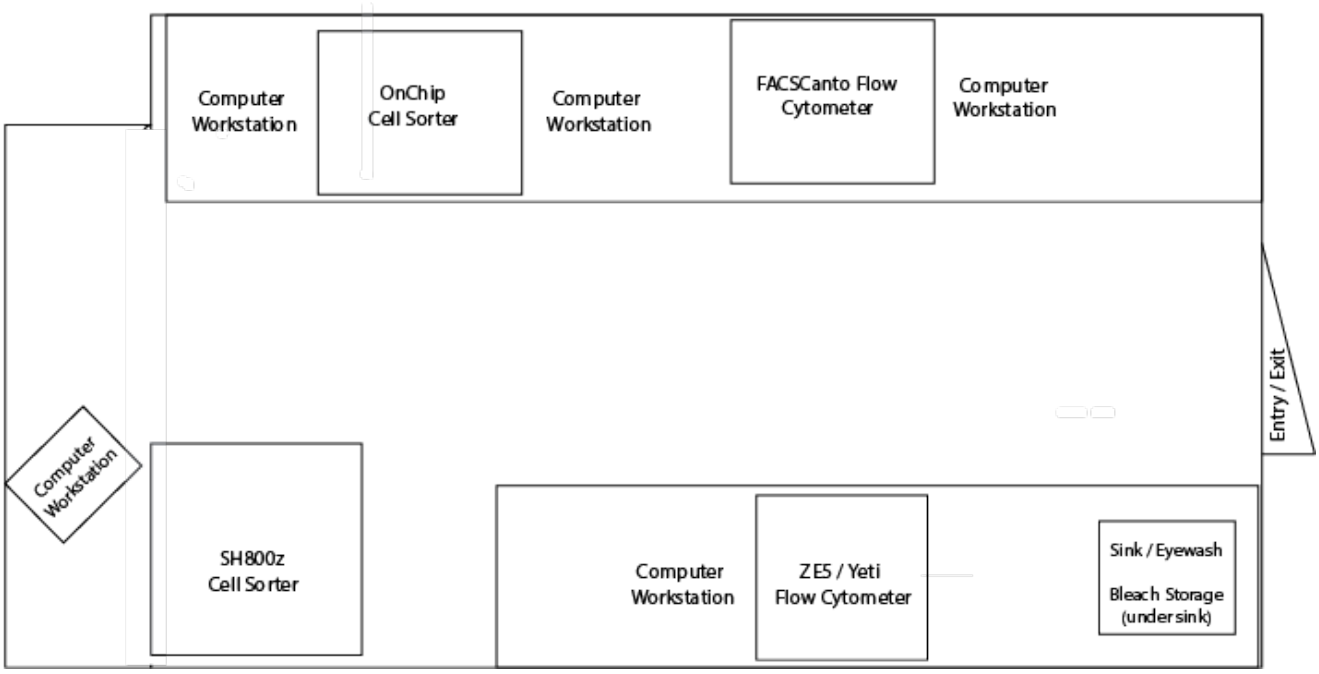
3.6.3.2. Secondary containers for carrying liquid waste containers are disinfected by spraying down with 70% ethanol or isopropyl alcohol, and may be autoclaved if needed.

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

Location Document
Title: New Science Building Laboratory, Rm 449
Location#: CCSL Location_4
Purpose: To provide safe handling procedures and operations for all personnel working in this facility.

The DART Cytometry & Cell Sorting Laboratory is a Biosafety Level 2 (BSL 2) certified facility that is located in the New Science Building, Room 449. The Cytometry Lab equipment in the room include a ZE5 flow cytometer, a SH800 cell sorter, a Canto flow cytometer, and an OnChip cell sorter. The ZE5, Canto and OnChip instruments in this facility does not pose a risk for creating aerosols, while the SH800 cell sorter may create aerosols.

4.1. Laboratory Facilities



4.1.1. The laboratory has one sink for hand washing directly to the left of the entrance. An eye wash station, emergency shower, spill kit, and fire extinguisher are located in 'Safety Havens.' The nearest one is down the hall to the left followed by an immediate right. Additional Safety havens are located down the halls to the left and right.

4.1.2. The eye wash station and emergency shower are maintained and inspected by NYULH facilities. Weekly inspection is completed by the Cytometry Lab staff and issues are reported to NYULH facilities.

4.1.3. The facility is cleaned and maintained by Collins Building Services (CBS), supplemented by the laboratory staff.

4.2. Restricted Access

4.2.1. Entry into the DART Cytometry & Cell Sorting Laboratory is restricted to authorized individuals who have received medical clearance from OHS, have taken the *Intro to Biosafety*

training, the *OSHA Bloodborne Pathogens* training on FOCUS and in-person, and reviewed the SOPs for the DART Cytometry & Cell Sorting Laboratory.

4.2.2. NYULH's Biosafety Officer/Specialist and Environmental Specialists will be granted access to conduct unannounced inspections.

4.2.3. Entry into the DART Cytometry & Cell Sorting Laboratory is restricted by an ID card reader. Only authorized NYULH personnel are permitted to enter. All others must be accompanied by a Cell Sorting Laboratory staff member.

4.3. Biosafety Cabinets (BSCs) and Aerosol Management Unit

4.3.1. There is one BCC300AMS Class II BSC that is maintained and operated by the Cytometry & Cell Sorting Lab located in the facility. The BCC300AMS Class II BSC houses the SH800Z Cell Sorter. Extra bench space for sample preparation is located in the tissue culture room next door.

Name	Room	Model	Serial No.
BCC300AMS Class II BSC	NSB 449	BCC300AMS	119130

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

4.3.3. **Aerosol Management Unit** –This unit serves to remove and filter aerosols from the cytometer's interior compartments. It is built into the BSC and connected via tubing to the back of the SH800Z Cell Sorter. The aerosol management unit is controlled via power buttons on the front control panel of the BSC.

4.4. Additional equipment

4.4.1. Flow Cytometers and Cell Sorters

Name	Room	Serial No.	SOP #
SH800 Cell Sorter	NSB 449	1800030	**CCSL SOP_107**
ZE5 Flow Cytometer	NSB 449	26	**CCSL SOP_106**
OnChip Cell Sorter	NSB 449	00062	**In progress**
FACSCanto Flow Cytometer	NSB 449	V0127	**In progress**

4.5. Entry to the DART Laboratory and Personal Protective Equipment (PPE)

4.5.1. All outside clothing not worn under a lab coat such as coats, hats, and so forth, are not permitted and should be secured outside of the facility before entering. Bags and anything not to be used in the Core Laboratory are subject to this as well.

4.5.2. It is the user's responsibility to have gloves with them when they enter the facility. The Core does not provide this resource to its satellite locations. Wearing gloves is required. They are disposed of when overtly contaminated and removed when work is completed or integrity is compromised. Gloves are not to be worn outside the lab.

4.5.3. Lab coats are the user's responsibility and are to be worn at all times while inside the lab. Non-disposable lab coats are laundered on a regular basis by NYULH Building Services.

4.5.4. The laboratory door should remain closed except when entering and exiting the lab.

4.6. Aerosol generating procedures

4.6.1. 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. As the ZE5 analyzer is not located in a biosafety cabinet, users conducting cell analysis must have prepared their samples completely ahead of time.

4.6.2. 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;
- Opening a package containing an infectious pathogen; and,
- Operation of the ZE5 analyzer or SH800 cell sorter (SOP#s specified above).

4.7. Exit out of the DART Cytometry and Cell Sorting Laboratory

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

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

4.7.3. Decontaminated liquid biohazard waste should be emptied into the sink and flushed with large amounts of tap water (Refer to 1.5 in **CCSL SOP_112** for proper liquid decontamination practices).

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

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

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

Standard Operating Procedures
Title: DART Cytometry and Cell Sorting Laboratory Standard Laboratory Practices for operation of the Skirball 3-9 **MoFlo XDP Cell Sorter**
SOP#: CCSL SOP_101
Purpose: To provide safe handling procedures and operations for all personnel working in the facility.

1. Materials

Item	Manufacturer	Catalog No.
FACSFlow Sheath	BD	342003
Glutaraldehyde-fixed Chicken Red Blood Cells (CRBCs)	Sigma	R0504
Align Flow Beads Red	Molecular Probes	A16504
Align Flow Beads Yellow/Green	Molecular Probes	C16509
Align Flow Beads UV	Molecular Probes	A16502

2. Use of MoFlo XDP Cell Sorter and Walker Class II BSC

2.1. Considering the containment measures in place for the Beckman Coulter (BCI) MoFlo XDP cell sorter, the sorter's use is not considered a high-risk procedure. However, in order to ensure optimal safety, certain procedures need to be followed during setup, use and shutdown of the instrument.

2.2. The BCI MoFlo XDP cell sorter is operated according to the manufacturer's manual (available at NYULH DART Cytometry and Cell Sorting Laboratory Facility at Skirball 3-9).

2.3. Startup Protocol

Instrument startup steps should be performed in the following order:

Step	Procedure	Comments
1	Turn on Walker BSC and BCI MoFlo cell sorter.	With the BSC front closed, including keeping the front access guard panel closed, turn BSC power switch to "Airflow On" or "Airflow + Light On." Remove front access guard panel.
2	After 1 minute of airflow, open front panel door of BSC and proceed with MoFlo setup.	Ensure that the BSC airflow alarm that is present briefly on startup shuts off, and that the green led lights near the power switch are illuminated, thus indicating safe airflow.
3	Check sheath, and waste levels.	Sheath should not be added unless waste tank is emptied. See section 2.6 for emptying waste. Add sheath and empty waste as necessary.

4	Turn on JunAir air compressor and check vacuum supply.	Ensure that the waste container gauge reads 5 – 15psi of vacuum, and that sheath supply tank gauge reads 30 or 60psi, depending on which nozzle is installed in the MoFlo.
5	Verify installed nozzle size; change to correct nozzle if needed.	
6	Turn stream on, and start Summit (MoFlo control) software.	Verify vacuum system is clearing waste stream.
7	Align and setup sorter as per the manufacturer's user manual.	
8	Establish proper side streams, and complete Intellisort-II setup.	
9	Change necessary optical filters.	Rear BSC hatches must be replaced after changing optical filters, and before running biohazardous samples.
10	Ensure BSC hatches are closed, airflow L.E.D.s indicate safe airflow and proper PPE is used.	
11	Begin running samples/sorting.	

These steps must be completed before a biohazardous sample is run on the instrument as some steps require open hatches on the hood and can create aerosols.

2.4. Clog or failure protocol

2.4.1. In the event of a clog, the stream may lose stability and generate an aerosol. The stream should be turned off immediately and the sample removed. If the stream restarts correctly, the sort can continue. If the clog persists, the nozzle must be removed and cleaned or replaced with a secondary stand-by nozzle.

2.4.2. If the nozzle must be removed for cleaning, it should be treated as biohazardous. After determining that the nozzle needs to be removed, the operator must wait 20 minutes before opening the BSC front panel door for any aerosol to clear from the interior compartments. The stream should be turned off, and the nozzle taken out of the machine. Within the BSC, the nozzle should be placed into a 5mL tube, filled with 10% Contrad detergent, which is then capped. This tube is then placed into the sonicator for cleaning. After sonication, the capped tube is opened within the BSC and the cleaned nozzle can be replaced.

2.4.3. Prevention of clogs by filtering all samples through a 70um cell strainer (or smaller) will greatly reduce the likelihood of aerosol generation, and is therefore required. Samples should be filtered prior to delivery to operator, though strainers will be available in the sorting room to re-strain particularly problematic samples.

2.4.4. In the event that the vacuum line, or vacuum pump fails, an aerosol can be created where the stream enters the waste catcher. Turn the stream off immediately and attempt to

resume house-supplied vacuum. If unable, the sorter should not be used until Facilities has resolved the issue.

2.5. Shutdown protocol

After sorting is completed, follow the protocol below for sort shutdown:

Steps	Procedure	Comments
1	Remove Collection tubes from sort chamber.	Wait at least 60 seconds after stream is turned off before opening sorting chamber in order to allow aerosol to settle.
2	Remove all samples and infectious material from sorter and hood.	All waste tubes should be capped and placed in a red biohazard waste bag. The waste bag should be sealed and sprayed before removal.
3	Run a tube of 10% bleach for 10 minutes and run the sample line decontamination procedure (section 2.5)	This will decontaminate all sample tubing.
4	Shutdown Moflo according to manufacturer manual.	
5	Wipe down all surfaces with 70% ethanol or 10% bleach.	Both surfaces inside and outside the hood should be decontaminated as in Section 2.7.
6	Empty waste tank if full.	Waste needs to be decontaminated before disposal. See section 2.6
7	Shutdown Junair Compressor.	
8	Turn off Walker BSC.	Replace front access guard panel with airflow on. Airflow alarm will sound, and BSC can be shutdown.

2.5.1. Sample line decontamination procedure

2.5.1.1. Load a tube filled with 10% bleach to a volume greater than that of the sample just run. Load and run the bleach through the sample lines for 5 minutes. Increase sample pressure either by pushing the "boost" button on the MoFlo pressure console, or by manually increasing sample pressure via the adjustment knob on the pressure console. Repeat above procedure with a tube of diH20 to rinse out the bleach. Lastly, repeat this procedure with a sample tube of 10% Contrad detergent in diH_2O.

2.5.1.2. This procedure ensures that ALL tubing that is exposed to sample during regular operation is disinfected. These surfaces include the sample tubing, the pinch valve tubing and surfaces in the flow body and nozzle.

2.6. Beckman Coulter MoFlo XDP cell sorter waste disposal

2.6.1. All material entering the sorter's waste tank should be considered biohazardous and must be disinfected before disposal down the sink in the cell sorting laboratory. To accomplish this, 300 - 400mL of Wescodyne disinfectant must be added to the waste tank before use. This ensures that the Wescodyne concentration will be high enough to

decontaminate fully when the tank is completely filled. Wescodyne solution must be in contact with contaminated waste fluid for a minimum of 30 minutes before disposal, after which the waste fluid can be disposed of in the lab sink. The inside of the tank should be visually inspected after emptying to confirm integrity has not been compromised.

2.7. Surface disinfection

2.7.1. After sorting all surfaces inside the hood and in the MoFlo's interior compartments should be disinfected with 70% ethanol.

2.7.2. Surfaces outside the hood that may have accidentally become contaminated should be decontaminated with 70% ethanol or 10% bleach. This includes computer desk surfaces, as well as the keyboard and mouse.

2.8. MoFlo fluidics decontamination

This procedure should be run on a monthly basis or when the instrument's fluidics have become contaminated.

1. Remove sheath filter holder, empty sheath fluid from filter and holder, and fill filter holder containing filter with 10% bleach. Re-install filter in MoFlo.
2. Fill sheath tank with 10% Bleach solution, empty waste tank
3. Re-pressurize the fluidics system, start stream.
4. Run 10% bleach for 15 minutes, cycling all valves to ensure they are cleaned appropriately. Run 10% bleach on the sample port during this wash.
5. Empty both the sheath and waste tanks, rinse with diH_2O – at least 4 changes of diH_2O for sheath tank.
6. Remove sheath filter holder, rinse filter and filter holder thoroughly with diH_2O – at least 4 changes of diH_2O. Fill sheath filter and holder with diH_2O and re-install sheath filter in MoFlo.
7. Run stream with diH_2O for 30 minutes. Change diH_2O in sheath tank. Run stream with diH_2O for 30 minutes. Run diH_2O on the sample port during these rinses.
8. Empty remaining diH_2O from sheath filter and holder, and sheath and waste tanks. Re-fill sheath filter holder, and sheath tank with sheath fluid.
9. Re-pressurize the fluidics system, and run sheath to eliminate air bubbles from the system.

2.9. Service

2.9.1. Field service engineers when working on the MoFlo XDP are required to abide by this SOP.

Standard Operating Procedures
Title: DART Cytometry and Cell Sorting Laboratory Standard Laboratory Practices for operation of the Skirball 3-9 **SY3200 Cell Sorter**
SOP#: CCSL SOP_102
Purpose: To provide safe handling procedures and operations for all personnel working in the facility.

1. Materials

Item	Manufacturer	Catalog No.
FACSFlow Sheath	BD	342003
SortCal Beads	Sony BioTech	700002

2. Use of SY3200 Cell Sorter and modified Baker SteriGard BSC

2.1. Considering the containment measures in place for the Sony SY3200 Cell Sorter, the sorter's use is not considered a high-risk procedure. However, in order to ensure optimal safety, certain procedures need to be followed during setup, use and shutdown of the instrument.

2.2. The Sony SY3200 cell sorter is operated according to the manufacturer's manual (available at NYULH DART Cytometry and Cell Sorting Laboratory Facility at Skirball 3-9).

2.3. Startup protocol

Instrument startup steps should be performed in the following order:

Step	Procedure	Comments
1	Check sheath, waste and diH20 levels.	Sheath should not be added unless waste tank is emptied. See section 2.6 for emptying waste. Add sheath and diH20 as necessary.
2	Ensure Air and Vacuum supply lines are functioning.	Ensure vacuum filter is dry. Replace if necessary.
3	Turn on BSC and Sony SY3200 cell sorter.	Machine and blower should be on for 15 minutes before use.
4	Turn on rinse stream and ensure it is flowing in waste catcher.	Verify vacuum system is clearing waste stream
5	Switch to sheath stream and ensure droplets are forming.	
6	Align and setup sorter as per the manufacturer's user manual.	

No sample should be placed on the sorter until these steps have been completed.

2.4. Clog and/or failure protocol

2.4.1. In the event of a clog, the stream may lose stability and generate an aerosol. The stream should be turned off immediately and the sample removed. Any aerosol should be contained within the sort chamber and filtered out by the Aerosol Management System (AMS). This can be engaged by pressing the AVAC button on the front of the cabinet. If

the stream restarts correctly, the sort can continue. If the clog persists, the nozzle must be removed and cleaned or replaced with a secondary stand-by nozzle**.**

2.4.2. If the nozzle must be removed for cleaning, it should be treated as biohazardous. After determining that the nozzle needs to be removed, the operator should activate the AMS and wait 60 seconds for any aerosol to clear from the interior compartments. The stream should be turned off, and the nozzle taken out of the machine. The nozzle should be placed into a 5mL tube, filled with 10% Contrad detergent, which is then capped. This tube is then placed into the sonicator for cleaning. After sonication, the capped tube containing the nozzle must not be opened until it is back in the BSC.

2.4.3. Prevention of clogs by filtering all samples through a 70um cell strainer (or smaller) greatly reduces the likelihood of aerosol generation, and is therefore required. Samples should be filtered prior to delivery to operator, though strainers will be available in the sorting room to re-strain particularly problematic samples.

2.4.4. In the event that the vacuum line fails, an aerosol can be created where the stream enters the waste catcher. Any aerosol should be contained within the sort block and filtered out by the AMS. Turn the stream off immediately and attempt to resume house-supplied vacuum. If unsuccessful, the sorter cannot be used until Facilities has resolved the issue.

2.5. Shutdown protocol

After sorting is completed, follow the protocol below for sort shutdown:

Step	Procedure	Comments
1	Remove Collection tubes from sort chamber.	Wait at least 60 seconds after stream is turned off before opening sorting chamber in order to allow aerosol evacuation.
2	Remove all sample and infectious material from sorter and hood.	All waste tubes should be capped and placed in waste bag. Waste bag should be sealed and sprayed before removal.
3	Run a tube of 10% bleach for 5 minutes and run the sample line decontamination procedure (section 2.5.1)	This will decontaminate all sample tubing
4	Wipe down all surfaces with 70% ethanol or 10% bleach.	Both surfaces inside and outside the hood should be decontaminated as in Section 2.7
5	Empty waste tank if full.	Waste needs to be decontaminated before disposal. See section 2.6
6	Shutdown software, SY3200, and air compressor.	

2.5.1. Sample line decontamination procedure

2.5.1.1. In the Sony SY3200 Software ensure the flow is switched from Sheath to Rinse. Load a tube filled with 10% bleach to a volume greater than that of the sample just run. Load and run the bleach through the sample lines. Toggle sample flow on and

off to ensure that the entire nozzle is disinfected. Repeat above procedure with a tube of diH20 to rinse out the bleach.

2.5.1.2. This procedure ensures that ALL tubing that is exposed to sample during regular operation is disinfected. These surfaces include the sample tubing, the pinch valve tubing and surfaces in the flow body and nozzle.

2.6. Sony SY3200 cell sorter waste disposal

2.6.1. All material entering the sorter's waste tank should be considered biohazardous and must be disinfected before disposal down the sink in the cell sorting laboratory. To accomplish this, ~1L of Wescodyne disinfectant must be added to the waste tank before use. This ensures that the Wescodyne concentration will be high enough to decontaminate fully when the tank is completely filled. Wescodyne solution must be in contact with contaminated waste fluid for a minimum of 30 minutes before disposal, after which the waste fluid can be disposed of in the lab sink. The inside of the tank should be visually inspected after emptying to confirm integrity has not been compromised.

2.7. Surface disinfection

2.7.1. After sorting all surfaces inside the hood and in the Synergy's interior compartments should be disinfected with 70% ethanol.

2.7.2. Surfaces outside the hood that may have accidentally become contaminated should be decontaminated with 70% ethanol or 10% bleach. This includes computer desk surfaces, as well as the keyboard and mouse.

2.8. SY3200 fluidics decontamination

This procedure should be run on a monthly basis or when the instrument's fluidics have become contaminated.

1. Remove sheath and diH_2O filters and replace with bypass connectors.
2. Fill sheath and diH_2O tanks with 10% Bleach solution.
3. Re-pressurize the fluidics and start stream with liquid form the rinse tank for 15 minutes.
4. Switch over to liquid from the sheath tank for 15 minutes.
5. Run 10% bleach sample line during steps 3 and 4, cycling all valves to ensure they are cleaned appropriately.
6. Empty both the sheath and waste tanks rinse with diH_2O.
7. Fill both tanks with diH_2O.
8. Run stream with diH_2O for 15 minutes from sheath tank and then 15 minutes from waste tank. Run diH_2O on the sample port during these rinses.
9. Refill diH_2O tank with diH_2O and the sheath tank with sheath fluid.
10. Replace sheath and diH_2O filters with new filters and prime system.

2.9. Service

2.9.1. Field service engineers when working on the SY3200 are required to abide by this SOP.

Standard Operating Procedures
Title: DART Cytometry and Cell Sorting Laboratory Standard Laboratory Practices for operation of the Skirball 3-9 **FACSAria IIu Cell Sorter**
SOP#: CCSL SOP_103
Purpose: To provide safe handling procedures and operations for all personnel working in the facility.

1. Materials

Item	Manufacturer	Catalog No.
FACSFlow Sheath	BD	342003
CST Beads	BD	655051
Accudrop Beads	BD	345249

2. Use of Self-Serve FACSAria IIu Cell Sorter and bioBUBBLE Benchtop Biocontainment Enclosure

2.1. Considering the containment measures in place for the Becton Dickenson FACSAria IIu Cell Sorter, the sorter's use is not considered a high-risk procedure. However, in order to ensure optimal safety, certain procedures need to be followed during setup, use and shutdown of the instrument.

2.2. The Self-Serve FACSAria IIu cell sorter is operated according to the manufacturer's manual (available at NYULH DART Cytometry and Cell Sorting Laboratory at Skirball 3-9).

2.3. The FACSAria IIu cell sorter can be operated as a user self-serve instrument ONLY by certified users that have been approved by the Cytometry Lab Staff and have read this SOP.

2.4. Startup protocol

Instrument startup steps should be performed in the following order:

Step	Procedure	Comments
1	Verify bioBUBBLE blowers are running and readings are adequate.	If blowers are off, the main power switch is located on the power entry module on the lower backside of the unit.
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	Check sheath, waste and other solution levels.	Refill or empty as necessary at this time. Add bleach to waste tank as per the **Decontamination and Chemical Use SOP (CCSL SOP_112).**
4	Run Fluidics Startup (if necessary) and turn stream on.	Startup only necessary on Mondays. Let stream stabilize and set gap and drop. Engage "sweet spot."
5	Run CST beads for instrument QC.	
6	Run Accudrop beads and perform drop delay setup.*	
7	Set up sort side streams for appropriate collection vessels.*	
8	Change necessary optical filters.*	

9	Ensure hood hatches are closed, hood pressure gauge is correct and proper PPE is used.	
10	Begin running samples/sorting.	

*** Steps 6, 7, and 8 must be completed *before* a biohazardous sample is run on the instrument. These steps require open hatches on the hood and can create aerosols.**

2.5. Clog of failure protocol

2.5.1. In the event of a clog, the stream may lose stability and generate an aerosol. If the stream restarts correctly, the sort can continue.

2.5.2. 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.

2.5.3. 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 instrument. Before removal from the hood it should be placed into a 15mL conical tube, filled with either diH2O 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 bioBUBBLE.

2.5.4. Prevention of clogs by filtering all samples through a 70um cell strainer (or smaller) greatly reduces the likelihood of aerosol generation, and is therefore required. Samples should be filtered prior to delivery to operator, though strainers will be available in the sorting room to re-strain particularly problematic samples.

2.6. Shutdown protocol

After sorting is completed, follow the protocol below for sort shutdown:

Step	Procedure	Comments
1	Remove Collection tubes from sort chamber.	Wait at least 60 seconds after stream is turned off before opening sorting chamber in order to allow aerosol evacuation.
2	Remove all sample and infectious material from sorter and hood.	All waste tubes should be capped and placed in waste bag. Waste bag should be sealed and sprayed before removal.
3	Run the sample line decontamination procedure (Section 2.6.1).	This will decontaminate all sample tubing.
4	Wipe down all surfaces with 70% ethanol or 10% bleach.	Both surfaces inside and outside the hood should be decontaminated as in Section 2.8.
5	Empty waste tank if full.	Waste needs to be decontaminated before disposal. See section 2.7.
6	Shutdown software, FACSAria IIu, and chilled water re-circulator.	

2.6.1. Sample line decontamination procedure

2.6.1.1. To decontaminate the FACSAria IIu, a tube of 10% Bleach should be run on the sample port for 5 minutes at high flow rate (11). Toggle sample flow on and off to

ensure that the entire nozzle is disinfected. Repeat above procedure with a tube of diH20 to rinse out the bleach.

2.6.1.2. On a daily basis (besides Fridays), run a 'Clean Flow Cell' (Cytometer>Cleaning modes>Clean Flow Cell) twice, using H2O as the cleaning solution. This will leave the flow cell and sample lines in H2O, preventing salt crystals from forming there.

2.6.1.3. On Fridays, the automated "Fluidics Shutdown" procedure of the FACSDiva Software can be initiated. This will flush the instrument with 70% EtOH. Using this procedure for shutdown will decontaminate the instrument and reduce salt buildup, helping to prevent future clogs.

2.7. Self-Serve FACSAria IIu cell sorter waste disposal

2.7.1. All material entering the sorter's waste tank should be considered biohazardous and must be disinfected before disposal down the sink in the cell sorting laboratory. To accomplish this, ~1L of Wescodyne disinfectant or 10% Bleach must be added to the waste tank before use. This ensures that the disinfectant concentration will be high enough to decontaminate fully when the tank is completely filled. Disinfectant solution must be in contact with contaminated waste fluid for a minimum of 30 minutes before disposal, after which the waste fluid can be disposed of in the lab sink. The inside of the tank should be visually inspected after emptying to confirm integrity has not been compromised. 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.

2.8. Surface disinfection

2.8.1. After sorting, all surfaces inside the bioBUBBLE and in the FACSAria IIu's interior compartments should be disinfected with 70% ethanol.

2.8.2. Surfaces outside the bioBUBBLE that may have accidentally become contaminated should be decontaminated with 70% ethanol or 10% bleach. This includes computer desk surfaces, as well as the keyboard and mouse.

2.9. Self-Serve FACSAria IIu fluidics decontamination

This procedure should be run on a monthly basis or when the instrument's fluidics have become contaminated.

1. Remove sheath and diH_2O filters and replace with bypass connectors.
2. Fill sheath tank with 10% Bleach solution.
3. Re-pressurize the fluidics and start stream for 15 minutes.
4. Run 10% bleach on sample line during step 3, cycling all valves to ensure they are cleaned appropriately.
5. Empty both the sheath and waste tanks rinse with diH_2O.
6. Fill sheath tank with diH_2O.
7. Run stream with diH_2O for 15 minutes from sheath tank. Run diH_2O on the sample port during this rinse.
8. Refill the sheath tank with sheath fluid.
9. Replace sheath and diH_2O filters with new filters and prime system.

2.10. Service

2.10.1. Field service engineers when working on the FACSAria IIu are required to abide by this SOP.

Standard Operating Procedures
Title: DART Cytometry & Cell Sorting Laboratory Standard Laboratory Practices for operation of the Bellevue CD645 **FACSAria IIu Cell Sorter**.
SOP#: CCSL SOP_104
Purpose: To provide safe handling procedures and operations for all personnel working with this instrument.

1. Materials

Item	Manufacturer	Catalog No.
FACSFlow Sheath	BD	342003
CST Beads	BD	655051
Accudrop Beads	BD	345249

2. Use of FACSAria IIu Cell Sorter and Baker BioProtect III BSC

2.1. Considering the containment measures in place for the Becton Dickenson FACSAria IIu Cell Sorter (see section 2.5), the sorter's use is not considered a high-risk procedure. However, in order to ensure optimal safety, certain procedures need to be followed during setup, use and shutdown of the instrument.

2.2. The FACSAria IIu cell sorter is operated according to the manufacturer's manual (available at NYULH DART Cytometry and Cell Sorting Laboratory Facilities at Bellevue CD643 and Skirball 3-9).

2.3. The FACSAria IIu cell sorter can be operated as a user self-serve instrument during specified hours ONLY by certified users that have been approved by the Cytometry Lab Staff and have read this SOP.

2.4. Startup protocol

Instrument startup steps should be performed in the following order:

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 our **Decontamination and Chemical Use SOP (CCSL SOP_112).**
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.**

2.5. Clog and/or failure protocol

2.5.1. 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.

2.5.2. 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.

2.5.3. 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 BSC it should be placed into a 15mL conical tube, filled with either diH2O or EtOH, which is then capped. This tube can then be taken out of the BSC and placed into the sonicator for cleaning. The tube should not be opened again until inside the BSC.

2.5.4. Prevention of clogs by filtering all samples through a 70um cell strainer (or smaller) greatly reduces the likelihood of aerosol generation, and is therefore required. Samples should be filtered prior to delivery to operator, though strainers will be available in the sorting room to re-strain particularly problematic samples.

2.6. Shutdown protocol

After sorting is completed, follow the protocol below for sort shutdown:

Step	Procedure	Comments
1	Remove Collection tubes from sort chamber.	Wait at least 60 seconds after stream is turned off before opening sorting chamber in order to allow aerosol evacuation.
2	Remove all sample and infectious material from sorter and hood.	All waste tubes should be capped and placed in waste bag. Waste bag should be sealed and sprayed before removal.
3	Run the sample line decontamination procedure (2.6.1)	This will decontaminate all sample tubing
4	Wipe down all surfaces with 70% ethanol or 10% bleach.	Both surfaces inside and outside the hood should be decontaminated as in Section 2.8.
5	Empty waste tank if full.	Waste needs to be decontaminated before disposal. See section 2.7.
6	Shutdown software, FACSAria IIu, Whisper unit, and chilled water re-circulator.	

2.6.1. Sample line decontamination procedure

2.6.1.1. To decontaminate the FACSAria IIu, a tube of 10% Bleach should be run on the sample port for 5 minutes at high flow rate (11). Toggle sample flow on and off to ensure that the entire nozzle is disinfected. Repeat above procedure with diH20 to rinse out the bleach.

2.6.1.2. On a daily basis (besides Fridays), run a 'Clean Flow Cell' (Cytometer>Cleaning modes>Clean Flow Cell) twice, using H2O as the cleaning solution. This will leave the flow cell and sample lines in H2O, preventing salt crystals from forming here.

2.6.1.3. On Fridays, the automated "Fluidics Shutdown" procedure of the FACSDiva Software can be initiated. This will flush the machine with 70% EtOH. Using this procedure for shutdown will decontaminate the instrument and reduce salt buildup, helping to prevent future clogs.

2.7. FACSAria IIu cell sorter waste disposal

2.7.1. All material entering the sorter's waste tank should be considered biohazardous and must be disinfected before disposal down the sink in the cell sorting laboratory. To accomplish this, ~1L of Wescodyne disinfectant or 10% Bleach must be added to the waste tank before use. This ensures that the disinfectant concentration will be high enough to decontaminate fully when the tank is completely filled. Disinfectant solution must be in contact with contaminated waste fluid for a minimum of 30 minutes before disposal, after which the waste fluid can be disposed of in the lab sink. The inside of the tank should be visually inspected after emptying to confirm integrity has not been compromised. 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.

2.8. Surface disinfection

2.8.1. After sorting all surfaces inside the BSC and in the FACSAria IIu's interior compartments should be disinfected with 70% ethanol.

2.8.2. Surfaces outside the BSC that may have accidentally become contaminated should be decontaminated with 70% ethanol or 10% bleach. This includes computer desk surfaces, as well as the keyboard and mouse.

2.9. Self-Serve FACSAria IIu fluidics decontamination

This procedure should be run on a monthly basis or when the machine's fluidics have become contaminated.

1. Remove sheath and diH_2O filters and replace with bypass connectors.
2. Fill sheath tank with 10% Bleach solution.
3. Re-pressurize the fluidics and start stream for 15 minutes.
4. Run 10% bleach on sample line during step 3, cycling all valves to ensure they are cleaned appropriately.
5. Empty both the sheath and waste tanks rinse with diH_2O.
6. Fill sheath tank with diH_2O.
7. Run stream with diH_2O for 15 minutes from sheath tank. Run diH_2O on the sample port

during this rinse.
8. Refill the sheath tank with sheath fluid.
9. Replace sheath and diH_2O filters with new filters and prime system.

2.10. Service

2.10.1. Field service engineers when working on the FACSAria IIu are required to abide by this SOP.

Standard Operating Procedures
Title: DART Cytometry & Cell Sorting Laboratory Standard Laboratory Practices for the ACLS West 329 **Cytoflex Flow Cytometer**.
SOP#: CCSL SOP_105
Purpose: To provide safe handling procedures and operations for all personnel working in the facility

1. Materials

Item	Manufacturer	Catalog No.
Cytoflex QC beads	Beckman Coulter	B53230

2. Use of Cytoflex Flow Cytometer

2.1. Any biohazardous or potentially biohazardous samples run on the Cytoflex must be handled according to standard BSL2 guidelines. In order to ensure the proper functioning of these instruments and prevent the potential for clogs or other operational issues, the proper protocols must be followed according to the available user guides and all cleaning and shutdown steps must be followed. The setup procedure outlined below will insure that certain biohazardous concerns are addressed.

Step	Procedure	Comments
1	Turn on the Cytoflex/ZE5 analyzer, and log into the computer.	Computer should always remain on.
2	Check sheath (diH_20), waste and other solution levels.	Refill or empty as necessary at this time. Add bleach to waste tank as per the **Decontamination and Chemical Use SOP (CCSL SOP_112).**
3	Open the software and initialize the instrument.	
5	Begin running samples.	

2.2. Clog of failure protocol

2.2.1. In the event of a clog, remove biohazardous sample and attempt to clear clog via software functions like Backflush, Prime, or Daily Clean.

2.2.2. Prevention of clogs by filtering all samples through a 70um cell strainer (or smaller) greatly reduces the likelihood of clogs, and is therefore required.

2.3. Shutdown protocol

After analysis is completed, follow the protocol below for shutdown:

Step	Procedure	Comments
1	Remove sample tube or plate from instrument and dispose of properly.	
3	Run the sample line decontamination procedure (Section 2.3.1).	This will decontaminate all sample tubing.
4	Wipe down all surfaces with 70% ethanol or 10% bleach.	
5	Empty waste tank if full.	Waste needs to be decontaminated before disposal. See section 2.4.

6	Shutdown software and Cytoflex.	Leave computer on!

2.3.1. Sample line decontamination procedure

2.3.1.1. To decontaminate the Cytoflex, select the Daily Clean procedure from the software (tube of 10% Bleach is run on the sample followed by a tube of diH20 to rinse out the bleach).

2.4. Cytoflex Flow Cytometer waste disposal

2.4.1. Waste from the Cytofelx is pumped into a 5 liter tank positioned to the left of the instrument. This tank must contain enough bleach (indicated by markings on the container) to render the final volume in the tank 10% bleach. Disinfectant solution must be in contact with contaminated waste fluid for a minimum of 30 minutes before disposal, after which the waste fluid can be disposed of in the lab sink. The inside of the tank should be visually inspected after emptying to confirm integrity has not been compromised.

2.5. Surface disinfection

2.5.1. After analysis, all surfaces around the Cytoflex should be disinfected with 70% ethanol or 10% bleach. This includes computer desk surfaces, as well as the keyboard and mouse.

2.6. Cytoflex fluidics decontamination

This procedure should be run on a monthly basis or when the instrument's fluidics have become contaminated.

1. Remove sheath and diH_2O filters and replace with bypass connectors.
2. Fill sheath tank with 10% Bleach solution.
3. Re-pressurize the fluidics and start stream for 15 minutes.
4. Run 10% bleach on sample line during step 3, cycling all valves to ensure they are cleaned appropriately.
5. Empty both the sheath and waste tanks rinse with diH_2O.
6. Fill sheath tank with diH_2O.
7. Run stream with diH_2O for 15 minutes from sheath tank. Run diH_2O on the sample port during this rinse.
8. Refill the sheath tank with sheath fluid.
9. Replace sheath and diH_2O filters with new filters and prime system.

2.7. Service

2.7.1. Field service engineers when working on the Cytoflex are required to abide by this SOP.

Standard Operating Procedures
Title: DART Cytometry & Cell Sorting Laboratory Standard Laboratory Practices for the ACLS West 329 and NSB 449 **ZE5 Flow Cytometers**.
SOP#: CCSL SOP_106
Purpose: To provide safe handling procedures and operations for all personnel working in the facility

1. Materials

Item	Manufacturer	Catalog No.
ZE5 QC beads	Propel Labs	PL00193

2. Use of ZE5 Flow Cytometers

2.1. Any biohazardous or potentially biohazardous samples run on the ZE5 must be handled according to standard BSL2 guidelines. In order to ensure the proper functioning of these instruments and prevent the potential for clogs or other operational issues, the proper protocols must be followed according to the available user guides and all cleaning and shutdown steps must be followed. 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 ZE5 analyzer, and log into computer.	Computer should always remain on.
2	Check sheath (diH_20), waste and other solution levels.	Refill or empty as necessary at this time. Add bleach to waste tank as per our **Decontamination and Chemical Use SOP (CCSL SOP_112).** The ZE5's sheath containers are automatically filled – refilling them is not necessary on this instrument.
3	Log into Everest account and begin the automated startup procedure.	The ZE5 goes through an automated startup, which prepares the instrument for use.
5	Begin running samples.	

2.2. Clog of failure protocol

2.2.1. In the event of a clog, remove biohazardous sample and attempt to clear clog via software functions like Probe Wash, Unclog, or running the cleaning experiment (see section 2.3.1).

2.2.2. Prevention of clogs by filtering all samples through a 70um cell strainer (or smaller) greatly reduces the likelihood of clogs, and is therefore required.

2.3. Shutdown protocol

After analysis is completed, follow the protocol below for shutdown:

Step	Procedure	Comments
1	Remove sample tubes or plate from instrument and dispose of properly.	
3	Run the cleaning procedure to decontaminate sample line (Section 2.3.1).	This will decontaminate all sample tubing.
4	Run software's Shutdown procedure.	May begin automatically if included in cleaning

		experiment.
5	Wipe down all surfaces with 70% ethanol or 10% bleach.	Both surfaces inside and outside the hood should be decontaminated as in Section 2.5.
6	Empty waste tank if full.	Waste needs to be decontaminated before disposal. See section 2.4.
7	Shutdown software.	Leave computer on.

2.3.1. Sample line decontamination procedure

2.3.1.1. To decontaminate the ZE5, a cleaning runlist should be used that runs a tube of 10% Bleach, followed by a tube of clenz, followed by diH2O, set to take up at least 300uL at 3.5 ul/sec. This runlist may include the automatic shutdown procedure.

2.4. ZE5 Flow Cytometer waste disposal

2.4.1. Waste from the ZE5 is pumped into one of two 5-liter tanks positioned inside the left cabinet of the instrument (red tops). Once one tank is full, the instrument automatically begins filling the other. Both tanks have level sensors that prevent overflow. This tank must contain enough bleach (indicated by markings on the container) to render the final volume in the tank 10% bleach. Disinfectant solution must be in contact with contaminated waste fluid for a minimum of 30 minutes before disposal, after which the waste fluid can be disposed of in the lab sink.

2.5. Surface disinfection

2.5.1. After analysis, all surfaces around the ZE5 should be disinfected with 70% ethanol or 10% bleach. This includes computer desk surfaces, as well as the keyboard and mouse.

2.6. ZE5 fluidics decontamination

This procedure should be run on a monthly basis or when the instrument's fluidics have become contaminated.

11. Remove sheath filter and replace with bypass connector.
12. Fill sheath tank with 10% Bleach solution.
13. Re-pressurize the fluidics and start stream for 15 minutes.
14. Run 10% bleach STAT tube during step 3, cycling all valves to ensure they are cleaned appropriately.
15. Empty both the sheath and waste tanks rinse with diH_2O.
16. Fill sheath tank with diH_2O.
17. Run stream with diH_2O for 15 minutes from sheath tank. Run diH_2O as a STAT tube during this step.
18. Run Shutdown Procedure.
19. Replace sheath filters with new filter and run Startup Procedure.

2.7. Service

2.7.1. Field service engineers when working on the ZE5 are required to abide by this SOP.

1. Materials

Item	Manufacturer	Catalog No.
SH800 Beads	Sony	LE-B3001

2. Use of SH800Z Flow Cytometer and Baker BCC300AMS Class II BSC

2.1. Considering the containment measures in place for the Sony SH800z Cell Sorter (see section 2.5), the sorter's use is not considered a high-risk procedure. However, in order to ensure optimal safety, certain procedures need to be followed during setup, use and shutdown of the instrument.

2.2. The SH800z cell sorter is operated according to the manufacturer's manual (available at NYULH DART Cytometry and Cell Sorting Laboratory Facilities at Skirball 3-9 and on our website).

2.3. The SH800z cell sorter can be operated as a user self-serve instrument during specified hours ONLY by certified users that have been approved by the Cytometry Lab Staff and have read this SOP.

2.4. Startup protocol

Instrument startup steps should be performed in the following order:

Step	Procedure	Comments
1	Turn on the Baker BSC blowers and lights, if necessary (BSC should always remain on).	The blowers must be on for 15 minutes before any human samples are run.
2	Turn on the SH800Z instrument, the air compressor, and computer.	The compressor must be on before any further action is taken.
3	Check sheath, waste and other solution levels.	Refill or empty as necessary at this time. Add bleach to waste tank as per our **Decontamination and Chemical Use SOP (CCSL SOP_112).**
4	Log into the pertinent account and begin the automated startup procedure, following the prompts on the screen.	This procedure covers the entire startup process, guides the user step by step, and prepares the instrument for use.
5	Change necessary optical filters.*	
6	Ensure hood hatches are closed, hood pressure gauge is correct and proper PPE is used.	
7	Begin running samples/sorting.	

*** Steps 4 and 5 must be completed *before* a biohazardous sample is run on the machine. These steps require open hatches on the hood and can create aerosols.**

2.5. Clog and/or failure protocol

2.5.1. 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 BSC's built-in Aerosol Management Unit. This unit can be turned up to full power for expedited evacuation of the sort chamber or in cases of a particularly bad clog. If Stream Breakoff control was maintained or once sort calibration has been repeated successfully, the sort can continue.

2.5.2. 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.

2.5.3. If the sort chip must be removed for replacement, it should be treated as biohazardous. The stream should be turned off and the chip ejected and removed from the machine, placed in its original packaging, and discarded in the appropriate biohazard trash receptacle.

2.5.4. Prevention of clogs by filtering all samples through a 70um cell strainer (or smaller) greatly reduces the likelihood of aerosol generation, and is therefore required.

2.6. Shutdown protocol

After sorting is completed, follow the protocol below for sort shutdown:

Step	Procedure	Comments
1	Remove Collection tubes from sort chamber.	Wait at least 60 seconds after stream is turned off before opening sorting chamber in order to allow aerosol evacuation.
2	Remove all sample and infectious material from sorter and hood.	All waste tubes should be capped and placed in waste bag. Waste bag should be sealed and sprayed before removal.
3	Run the sample line decontamination procedure (section 2.6.1)	This will decontaminate all sample tubing
4	Wipe down all surfaces with 70% ethanol or 10% bleach.	Both surfaces inside and outside the hood should be decontaminated as in Section 2.8.
5	Empty waste tank if full.	Waste needs to be decontaminated before disposal. See section 2.7.
6	Shutdown software, SH800z and compressor.	BSC should remain on.

2.6.1. Sample line decontamination procedure

2.6.1.1. To decontaminate the SH800z, follow the software directions for the Bleach clean (tube of >12mL 10% Bleach is run) followed by shutdown rinse (tube of >12mL diH2O is run) procedures.

2.6.1.2. The surfaces of the SH800Z and Baker BCC300AMS Class II BSC should be wiped down with 70% EtOH or 10% bleach. Blowers should run for at least 15 minutes after cabinet has been wiped down.

2.7. SH800z cell sorter waste disposal

2.7.1. All material entering the sorter's waste tank should be considered biohazardous and must be disinfected before disposal down the sink in the cell sorting laboratory. Waste from the

SH800Z 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. Disinfectant solution must be in contact with contaminated waste fluid for a minimum of 30 minutes before disposal, after which the waste fluid can be disposed of in the lab sink. The tank cap is equipped with a biohazard filter that allows air pressure release, but prevents aerosol from escaping. The filter is to be changed on a during the 6-month preventative maintenance.

2.8. Surface disinfection

2.8.1. After sorting all surfaces inside the BSC and in the SH800z's interior compartments should be disinfected with 70% ethanol.

2.8.2. Surfaces outside the BSC that may have accidentally become contaminated should be decontaminated with 70% ethanol or 10% bleach. This includes computer desk surfaces, as well as the keyboard and mouse.

2.9. Self-Serve SH800z fluidics decontamination

2.9.1. This procedure should be run on a monthly basis or when the machine's fluidics have become contaminated.

2.9.2. In the Maintenance section of the software, run the Ethanol cleaning procedure and follow the onscreen directions.

2.10. Service

2.10.1. Field service engineers when working on the SH800z are required to abide by this SOP.

Standard Operating Procedures
Title: DART Cytometry and Cell Sorting Laboratory Standard Laboratory Practices for Aerosol Containment Testing
SOP#: CCSL SOP_108
Purpose: To provide safe handling procedures and operations for all personnel working in the facility.

1. Materials

Item	Manufacturer	Catalog No.
GloGerm Beads (Oil)	GloGerm	GGO8O
MegaLite Pump	Environment Monitoring Systems	120190
Cyclex-d Cassettes	Environment Monitoring Systems	120135

2. In order to ensure containment of aerosols by the biosafety cabinets and other enclosures, the aerosol containment testing protocol should be used at a minimum after service of the biosafety cabinet or enclosure containing a cell sorter.

3. The equipment used in this protocol include a fluorescence microscope, and Glo Germ™ beads, as well as an E-Lite Pump™ and an Air-O-Cell™ cassette, both supplied by EMSL Analytical, Inc. The test protocol is adapted from the sampling guide available from the manufacturer. The protocol follows:

    1. Prior to sampling, calibrate the pump to 15 liters per minute.
    2. Remove and retain tape seal covering Air-O-Cell™ inlet and outlet.
    3. Attach the outlet (round hole) to the supplied tubing adapter, positioning the inlet within 1 foot of the sort chamber. The sort chamber cover should be removed.
    4. Begin sorting Glo Germ™ beads at 20,000 events per second and simulate an aerosol causing clog by moving the waste catcher.
    5. Start the sampling pump, and sample for 10 minutes.
    6. Remove Air-O-Cell™ from tubing, and reseal with the original tape. Label sample. For a positive control, the above steps should be repeated with the aerosol containment tubing pinched closed.
    7. Extract coverslip from inside of cassette, lay on slide and image using the fluorescent microscope. Count Glo Germ™ beads seen.

4. Aerosol containment is considered maintained when less than 2 beads are present per coverslip. Ensure that the positive control is run AFTER the test sample.

Standard Operating Procedures
Title: DART Cytometry and Cell Sorting Laboratory Standard Laboratory Practices for Tissue Culture Bio-Safety Cabinets
SOP#: CCSL SOP_109
Purpose: To provide safe handling procedures and operations for all personnel working in the facility.

1. Use of tissue culture biosafety cabinets

1.1. 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.

1.2. 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.

1.3. A biohazard bag should be present in the cabinet. Absorbent material (such as a dry clean room 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.

1.4. Liquid waste should be put into a dedicated container inside the BSC with sufficient bleach to achieve a final concentration of 10% and allowed to react for a minimum of 30 minutes 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.

1.5. 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.

1.6. Contaminated pipettes should be disposed of in the biohazard bags.

1.7. Anything removed from the BSC during the work session is to be decontaminated by wiping with 70% isopropyl alcohol while still in the BSC.

1.8. 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 within the BSC.

1.9. 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.

1.10. A fresh pair of outer gloves is donned and the hood is now wiped down completely with 70% isopropyl alcohol.

1.11. 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.

Standard Operating Procedures
Title: DART Cytometry and Cell Sorting Laboratory Standard Laboratory Practices for Centrifuges
SOP#: CCSL SOP_110
Purpose: To provide safe handling procedures and operations for all personnel working in the facility.

1. Centrifuges

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

1.1. Rotor buckets and lids shall be sprayed with 70% isopropyl alcohol and placed in the BSC or enclosure prior to loading.

1.2. Samples shall be loaded into rotor/rotor buckets and sealed with the cap for the rotor bucket while in BSC or enclosure.

1.3. After centrifuging, rotor/rotor buckets shall be moved to BSC or enclosure to unload samples. Samples shall **not** be unloaded in the open room.

1.4. Centrifuge and rotor chambers shall be disinfected with 70% isopropyl alcohol soaked wipers following use.

1.5. Prior to maintenance, equipment must be decontaminated.

Standard Operating Procedures
Title: DART Cytometry and Cell Sorting Laboratory Standard Laboratory Practices for CO_2 Incubators
SOP#: CCSL SOP_111
Purpose: To provide safe handling procedures and operations for all personnel working in the facility.

1. CO_2 Incubators

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

1.1. Flasks and culture plates shall be carried to and from the incubator using plastic secondary containers.

1.2. In the event of bacterial or fungal contamination in the incubators, flasks and culture plates shall be moved to a BSC. Shelves shall be wiped down with 70% isopropyl alcohol and shelves should be sterilized in an autoclave.

1.3. Gloves must be worn when handling cultures.

1.4. Prior to maintenance, equipment must be decontaminated.

Standard Operating Procedures
Title: DART Cytometry and Cell Sorting Laboratory Standard Laboratory Practices for Decontamination and Chemical Use
SOP#: CCSL SOP_112
Purpose: To provide safe handling procedures and operations for all personnel working in the facility.

1. 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:

1.1. Bench tops and external equipment surfaces: Work surfaces are wiped down with 70% ethanol or 10% bleach.

1.2. Water baths: Water baths are completely emptied of water and wiped down with 70% ethanol or 10% bleach.

1.3. Biosafety cabinet work surfaces: BSC work surfaces are sprayed 70% ethanol or 10% bleach.

1.4. Interior surfaces of equipment: Interior surfaces of centrifuges (including centrifuge buckets), incubators and other large equipment are wiped down with 70% ethanol or 10% bleach. Equipment is to be decontaminated prior to maintenance

1.5. Liquid Waste: Liquid biohazard waste will be decontaminated with sufficient bleach to achieve a final concentration of 10% for a minimum of 30 minutes and then emptied into the sink.

1.6. 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 potentially infectious material will be decontaminated by spraying with 70% ethanol before being laundered or discarded.

1.7. 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 70% ethanol or 10% bleach before transporting out of the lab.

2. Use of Chemicals

2.1. The same practices and training requirements will apply to the use of chemicals as in all other laboratories of NYULH. Specifically, personnel must be current with *Chemical Hygiene* and *Hazardous Waste* training requirements. EH&S offers training on the 2nd Thursday of each month.

2.2. For all chemicals used in the facility, the user must give the Laboratory Manager a corresponding Safety Data Sheet (SDS). 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.

3. Disposal of hazardous chemicals

3.1. 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.

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

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

Standard Operating Procedures
Title: Shipping and Receiving Infectious Substances and On-campus Transportation of Biological Samples
SOP#: CCSL SOP_113
Purpose: 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 course: *Shipping Hazardous Materials*, which is available at:

Focus can be accessed via at NYULMC.org.

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

2. On-campus Transportation of Biological Samples

2.1. General Notes

2.1.1. All samples and containers must have biohazard labels.

2.1.2. Avoid crowded areas whenever possible.

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

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

2.2. Packaging Instructions

2.2.1. 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).*

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

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

2.2.4. 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#: CCSL SOP_114
Purpose: To provide safe procedures for handling medical and facility emergencies

1. 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 NYULH's main number for Facilities: (212) 263 5275.

2.3. The building's emergency power generator should mitigate any loss of power to the essential containment equipment, allowing for 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. If possible secure any infectious material. Exit from the laboratory following the exit procedures listed in the appropriate **CCSL Location** document 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 the appropriate **CCSL Location** document 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 the appropriate **CCSL Location** document, 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 SOP **CCSL SOP_112_Decontamination and Chemical Use.**

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.

Standard Operating Procedures
Title: Exposure Incidents and Reporting
SOP#: CCSL SOP_115
Purpose: 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 at least 5 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
Occupational Health Services	212-263-5020	1 Park Avenue on the 3rd Floor	M-F 8:00AM-5:00PM
NYULH Emergency Department	212-263-5550	530 First Avenue, HCC 102	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 DART Cytometry and Cell Sorting Laboratory should be considered contaminated unless known otherwise.**

For work involving HIV-infected or potentially infected products, the worker must be escorted directly to the emergency room for **immediate** evaluation and counseling with regard to the risk of infection. **Post-exposure prophylaxis (PEP) should be offered according to the latest 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 NYULH's *OSHA Bloodborne Pathogens* training on FOCUS and in-person.

3. Reporting

Exposure incidents must be reported immediately either in person or by phone to an DART Cytometry Manager, the Director of the DART Cytometry Laboratory, and Occupational Health Services. Use **Facility Accident Report Form**, (Form **CCSL Form_F101** – a copy is at the end of this safety manual or can be obtained from the Laboratory Manager or Director) to document the incident.

Exposure incidents involving recombinant or synthetic DNA will be reported to the Institutional Biosafety Committee (IBC) after the above steps have been satisfied.

Standard Operating Procedures
Title: Spill Response and Reporting
SOP#: CCSL SOP_116
Purpose: To provide safe procedures for spill response in the facility

1. Materials

Item	Manufacturer	Catalog No.
Biohazard Bags	Lab Guard	19075388E
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. For chemical spills, use the spill kit to clean up the spill. The spill kit contains absorbent packets and pads. For bio-hazardous, non-chemical hazard spills decontaminate with bleach or other disinfectant and paper towels or absorbent pads can be used.

3.2. Procedure

1. Don a lab coat, two pairs of gloves, and eye or face protection.
2. Carefully cover the entire spill with an absorbent.
3. Taking care to avoid splashing pour a freshly prepared 1 in 10 dilution of bleach around the edges of the spill.
4. Allow a 30 minute contact time.
5. Pick up any glass with tongs.
6. Use dry clean room wipers or the absorbent pads to wipe up the spill working from the edges into the center.
7. Disinfect the spill area by spraying thoroughly with or 10% bleach, allowing a 10 minute contact time before wiping dry.
8. Discard waste and any contaminated PPE in a red biohazard bag.
9. Wash hands.

4. Reporting

Spills or accidents will be reported to the EH&S, the Laboratory Manager, and the Director of the DART Cytometry Laboratory. Fill out the **Facility Accident Report Form**, (Form **CCSL Form_F101** – a copy is at the end of this safety manual or can be obtained from the Laboratory Manager or Director) to document large spills or other potentially serious accidents.

Form CCSL - F101. 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 facility)? _____
6. Describe circumstances that lead to incident (work being done at that time, location of spill, equipment involved):_____
   _____
   _____
   _____
   _____
   _____
7. Other persons in the 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?

_____

_____

_____

a. Emergency Dept ____NYULH _____Bellevue HC ____No
b. Occupational Health Services ____Yes ____No
c. If any other medical/healthcare treatment was obtained outside the work-site, where was it obtained:

_____

_____

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

_____

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