SAFETY MANUAL AND STANDARD OPERATING PROCEDURES

FOR THE OCS Flow Cytometry Core

NEW YORK UNIVERSITY SCHOOL OF MEDICINE

Old Bellevue C&D building, Room 645

Revised: July 2016
<table>
<thead>
<tr>
<th>Version:</th>
<th>001</th>
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<tbody>
<tr>
<td>Approval Date:</td>
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<td>Approved by:</td>
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<tr>
<td>Peter Lopez</td>
<td>Director</td>
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<tr>
<td>Mark Olmsted</td>
<td>Environmental Specialist II, Environmental Health and Safety</td>
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<td>Written by:</td>
<td></td>
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<tr>
<td>Michael Gregory</td>
<td>Technical Director</td>
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<td>Mark Olmsted</td>
<td>Environmental Specialist II, Environmental Health and Safety</td>
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### List of Key Personnel

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
<th>Extension</th>
<th>Mobile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peter Lopez</td>
<td>Director</td>
<td>x30635</td>
<td>646-357-0168</td>
</tr>
<tr>
<td>Michael Gregory</td>
<td>Technical Director</td>
<td>x35097</td>
<td>516-641-5185</td>
</tr>
<tr>
<td>Kamilah Ryan</td>
<td>Asst Research Scientist</td>
<td>x35907</td>
<td>917-715-5337</td>
</tr>
<tr>
<td>Yulia Chupalova</td>
<td>Asst Research Scientist</td>
<td>x35907</td>
<td>203-570-3649</td>
</tr>
<tr>
<td>Colin Zollo</td>
<td>Asst Research Scientist</td>
<td>x35907</td>
<td>203-430-8189</td>
</tr>
</tbody>
</table>

### Important Telephone Numbers

**Note:** In an emergency, Communications can page personnel from key departments

<table>
<thead>
<tr>
<th>Note</th>
<th>Tel.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any Medical Center Emergency</td>
<td>x33911</td>
</tr>
<tr>
<td>Building Services</td>
<td>x35071</td>
</tr>
<tr>
<td>Communications</td>
<td>x37403</td>
</tr>
<tr>
<td>NYULMC Emergency Room (ER)</td>
<td>x35550</td>
</tr>
<tr>
<td>Bellevue Emergency Department (ED)</td>
<td>(212) 572-5082</td>
</tr>
<tr>
<td>Occupational Health Services (OHS)</td>
<td>x35120</td>
</tr>
<tr>
<td>Environmental Health and Safety (EH&amp;S)</td>
<td>x35159</td>
</tr>
<tr>
<td>Mark Olmsted, Biosafety Specialist</td>
<td>x35161</td>
</tr>
<tr>
<td>Sayra St. Omer, Biosafety Specialist</td>
<td>x10593</td>
</tr>
<tr>
<td>Marta Figueroa, Associate Director</td>
<td>x33887</td>
</tr>
<tr>
<td>NYULMC Facilities Management</td>
<td>x35275</td>
</tr>
<tr>
<td>Bellevue Facilities Management</td>
<td>(212) 562-4779</td>
</tr>
<tr>
<td>Poison Control</td>
<td>(212) 764-7667</td>
</tr>
<tr>
<td>Radiation Safety</td>
<td>x36888</td>
</tr>
<tr>
<td>Security</td>
<td>x73000</td>
</tr>
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</table>
HIV Core Laboratory Layout
1. Background

1.1. General Information
The BSL-2 cell-sorting laboratory is located on the 6th floor of the C&D Building of Bellevue Hospital. A locking door restricts access to this facility. The main laboratory room contains two class IIB re-circulating biosafety cabinets, one housing the cell sorting instrument and one for sample preparation/tissue culture. Special instrumentation is employed in this area to contain potential aerosols and reduce operator exposure. For more detailed information please see the CDC web site: http://www.cdc.gov/od/ohs/biosfty/biosfty.htm

2. Risk to Lab Personnel

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

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

2.2. High-speed Cell Sorting
High speed droplet based cell sorters can generate large amounts of aerosols, and recently published standards now specify a much higher level of biocontainment for cell sorting of unfixed human cells or other potentially biohazardous samples than have been traditionally followed. “If aerosol containment is incomplete, the safety features of the cell sorter must be modified such that no escape of aerosol can be detected. Alternately, sorters can be placed inside a biosafety containment cabinet” (Ref: I Schmid et al., International Society for Analytical Cytology Biosafety Standard for Sorting of Unfixed Cells. Cytometry Part A, 71A:414-437 (2007)).
Sorting of samples that represent potential toxic or infectious exposures via the aerosol route therefore require special procedures and laboratory conditions. This is true even for agents that are normally handled under standard BSL-2 laboratory conditions, such as primary human cell suspensions or cell lines. The heightened concern in the case of cell sorting arises from the possibility that cells or microorganisms may be delivered directly into the lungs of personnel in the vicinity of a cell sorter. In theory, this could increase the risk of infection with an occult pathogen, transfer of genetic material, sensitization to antigens or other potentially harmful effects. Although such adverse effects have not been documented as a consequence of exposure to aerosols during cell sorting, there is sufficient concern about this to warrant the implementation of procedures to eliminate any excess risk to personnel.

3. Containment

3.1. Facility Layout

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

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

3.2. Laboratory Facilities

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

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

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

3.3. Biosafety Cabinets (BSCs) and Aerosol Management Unit

3.3.1. There is one four foot Nuaire Class II BSC, and one Baker Bio Protect 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.

<table>
<thead>
<tr>
<th>Name/ Room#</th>
<th>Room</th>
<th>Model</th>
<th>BSC Serial No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baker BioProtect III BSC</td>
<td>CD645</td>
<td>BioProtect III</td>
<td>102073</td>
</tr>
<tr>
<td>Nuaire BSC</td>
<td>CD645</td>
<td>Nu-425-400</td>
<td>14398 ST</td>
</tr>
</tbody>
</table>

3.3.2. 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.

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

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

4. Facility Entry and Exit
   4.1. Refer to SOP FLOW-101

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

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

6. Medical Requirements, Surveillance, and Responses to Exposure

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

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

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

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

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

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

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

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

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

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

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

Title: Standard Laboratory Practices for the OCS Flow Cytometry Core Laboratory
SOP#: FLOW-101
Purpose: To provide safe handling procedures and operations for all personnel working in the facility

1. Materials

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

2.1. Entry into the OCS Flow Cytometry Core 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 OCS Flow Cytometry Core Laboratory.

2.2. NYULMC’s Biosafety Officer/Specialist and Environmental Specialists will be granted access to conduct unannounced inspections.

2.3. Entry into the OCS Flow Cytometry Core is restricted by a keyed lock and users must always be accompanied by a member of the Flow Core Staff unless otherwise authorized.

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

3. General Facility Requirements

3.1. Use of needles and other sharp instruments will not be used when biohazardous samples are present.

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

3.3. No food or drinks are allowed.

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

3.5. No jewelry (other than wedding bands) is to be worn under gloves.
3.6. No mouth pipetting is allowed in the facility.

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

3.8. Post-sort clean up should follow procedures listed in the Decontamination and Exit out of the OCS Flow Cytometry Core sections of this SOP.

3.9. Equipment is to be decontaminated prior to maintenance

4. Reagents and Supplies
4.1. All samples that are transported to the OCS Flow Cytometry Core must be contained using approved secondary containers. Refer to SOP FLOW-104

4.2. Unopened, non-infectous, non-toxic reagents and supplies are stored in rooms CD643 or CD647.

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

5.1. All outside clothing not worn under a lab coat must be left in room CD643 (office). Bags and anything not to be used in the Core Laboratory should be left there as well.

5.2. Wearing two pairs of gloves is required. They are disposed when overtly contaminated and removed when work is completed or integrity is compromised. Small, medium and large gloves are available to the left of the entrance to the room, and should be worn at all times. They will be sprayed with 70% ethanol or isopropyl alcohol as necessary and are not to be worn outside the lab.

5.3. Lab coats are available on a coat rack behind the door and are to be worn at all times while inside the lab. If a different size is needed, coveralls or surgical gowns can be supplied. Non-disposable lab coats are laundered on a regular basis by NYULMC Building Services.

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

6. Aerosol generating procedures

All transfers of biohazardous materials from one container to another container must take place within a BSC. Such transfers may not take place on the open bench. All other procedures that could generate aerosols must also be conducted in a BSC. The following are examples of these procedures:

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

7. Use of FACSaria Flow Cytometer and Baker BioProtect III BSC

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

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

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

7.2. In the event of a clog the stream may lose stability and generate an aerosol. In most cases, any aerosol should be contained within the sort block and filtered out by the Whisper Aerosol Management Unit. If the stream restarts correctly, the sort can continue.

7.2.1. If the sort block needs to be opened, or the collection containers removed after a clog, the operator must wait for 20 minutes before proceeding, to allow any aerosol to settle.

7.2.2. If the nozzle must be removed for cleaning, it should be treated as biohazardous. The stream should be turned off, and the nozzle taken out of the machine. Before removal from the hood it should be placed into a 15mL conical tube, filled with either 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 hood.

7.3. After sorting is complete the operator must wait for 5 minutes before removal of the collection apparatus. Tubes should be capped and plates covered. All collection containers should be labeled and wiped down with RelyOn Disinfectant Wipes before removal from the Biosafety cabinet.
7.4. To decontaminate the FACSARia, a tube of 70% EtOH should be run on the sample port for 5 minutes at high speed (10). Afterwards, one of two built-in automated procedures must be used.

7.4.1. If the machine is to be used again, the automated “Prepare for Aseptic Sort” procedure of the FACSDiva Software can be initiated. This procedure will flush all lines that come in contact with sample with 70% EtOH and disinfectant. Once complete the machine will be flushed with clean FACSFlow Sheath solution.

7.4.2. If the machine is no longer needed for the day, the automated “Fluidics Shutdown” procedure of the FACSDiva Software can be initiated. This will also flush the machine with 70% EtOH and disinfectant, but will not flush the machine with Sheath solution. Using this procedure for shutdown will reduce salt buildup and help prevent future clogs.

7.5. The surfaces of the FACSARia and Baker BioProtect Cabinet should be wiped down with 70% EtOH or RelyOn Disinfectant Wipes. Blowers should run for at least 15 minutes after cabinet has been wiped down.

7.6. Waste from the FACSARia is pumped into a 10 liter tank on the fluidics cart below the hood. This tank must contain enough bleach (1 liter) to render the final volume in the tank 10% bleach. The tank is capped with a biohazard filter that allows air pressure release, but prevents aerosol from escaping. The filter is to be changed on a monthly basis.

8. **Use of Nuaire Class II Type A BSC**

8.1. Before working in the BSC, the blowers and fluorescent light are switched on, and a biohazard bag, a spray bottle of 70% isopropyl alcohol, wipers, and pre-saturated wipes are placed in the cabinet. The blowers must be left on for 15 minutes before use.

8.2. All materials needed to complete the experiment are placed in the cabinet to limit the number of times hands pass through the air barrier. Equipment is not to be placed on the intake grills at the front of the cabinet, nor blocking the exhaust opening at the back of the cabinet.

8.3. A biohazard bag should be present in the cabinet. Absorbent material (such as a dry cleanroom wiper) is placed in the bottom of the biohazard bag. This bag is used for discarding solid waste (gloves, plastic waste, pipette tips). Once the bag is full, it is closed, wiped with 70% isopropyl alcohol and taken out of the cabinet to be collected into a larger covered waste container next to the cabinet.

8.4. Liquid waste should be put into a dedicated container inside the BSC with sufficient bleach to achieve a final concentration of 10% (0.5% sodium hypochlorite) and allowed to react for a minimum of 30 to 60 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.

8.5. Vacuum waste flasks should contain enough bleach to result in a 10% solution. They should never be filled more than 50%. An in-line vacuum filter must be present between the flask and the vacuum source.

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

8.7. Anything removed from the BSC during the work session is to be decontaminated by wiping with 70% isopropyl alcohol while still in the BSC.
8.8. At the end of each work session, culture tubes, racks and other material to be removed from the cabinet are decontaminated by wiping with 70% isopropyl alcohol while still within the BSC.

8.9. The wipers used during cleaning along with the outer gloves are placed into a biohazard bag while still within the BSC. Wipe or spray the outside of the bag with 70% isopropyl alcohol. Place the bag into a larger covered biohazard waste container next to the cabinet.

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

8.11. All tissue or cell culture related materials should be disposable whenever possible. Only disposable plastic pipettes and plastic tubes are to be used in the facility.

9. CO₂ Incubators
The following is a list of safety practices and procedures for doing work involving the use of cell culture incubators.

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

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

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

9.3. Gloves must be worn when handling cultures.

9.4. Prior to maintenance, equipment must be decontaminated.

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

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

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

10.3. After centrifuging, rotor/rotor buckets shall be moved to BSC to unload samples. Samples shall NOT be unloaded in the open room.

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

10.5. Prior to maintenance, equipment must be decontaminated.
11. Decontamination

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

Decontaminate as follows:

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

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

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

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

11.5. **Liquid Waste:** Liquid biohazard waste will be decontaminated with sufficient bleach to achieve a final concentration of 10% (0.5 % sodium hypochlorite) for a minimum of 30 to 60 minutes and then emptied into the sink.

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

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

12. Use of Chemicals

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

12.2. For all chemicals used in the facility, the user must give the Laboratory Manager a corresponding **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.

13. Disposal of hazardous chemicals

13.1. Hazardous chemicals will be collected in properly labeled containers in a designated area in the lab. Arrangements for the disposal of hazardous chemical waste may be made by contacting EH&S.

13.2. Biohazard waste **cannot** be discarded through the Hazardous Waste Disposal Program.
13.3. Arrangements for the disposal of hazardous chemical waste that is also a biohazard may be made by contacting EH&S.

14. Exit out of the OCS Flow Cytometry Core

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

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

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

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

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

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

<table>
<thead>
<tr>
<th>Item</th>
<th>Manufacturer</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biohazard Bags</td>
<td>Lab Guard</td>
<td>19075388E</td>
</tr>
<tr>
<td>RelyOn disinfectant cleaner</td>
<td>Dupont</td>
<td>Fisher # 19-120-3881</td>
</tr>
<tr>
<td>Blue absorbent pads</td>
<td>Fisherbrand</td>
<td>14-206-62</td>
</tr>
<tr>
<td>Spill Kit</td>
<td>Spill Defense</td>
<td>25916</td>
</tr>
</tbody>
</table>

2. Spill Response
   2.1. Spills will be decontaminated promptly by the responsible party.
   2.2. Personnel in the immediate area will be alerted and access to the contaminated area (around the spill) will be clearly marked with the biohazard floor sign and restricted.

3. Spill Clean-Up
   3.1. Don a lab coat, two pairs of gloves, and eye or face protection
   3.2. Use the spill kit to clean up the spill. The spill kit contains absorbent packets and pads.
   3.3. Pick up any glass with tongs
   3.4. Carefully cover the spill with the absorbent packets.
   3.5. Taking care to avoid splashing pour a freshly prepared 1 in 10 dilution of bleach around the edges of the spill.
   3.6. Allow a 30 to 60 minutes contact time.
   3.7. Use dry cleanroom wipers or the absorbent pads to wipe up the spill working from the edges into the center.
   3.8. Disinfect the spill area by spraying thoroughly with RelyOn disinfectant/cleaner, allowing a 10 minute contact time before wiping dry.
   3.9. Discard waste and any contaminated PPE in a red biohazard bag.
   3.10. Wash hands.
   3.11. Report spills and accidents to Environmental Health & Services.

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

<table>
<thead>
<tr>
<th>Department</th>
<th>Phone Number</th>
<th>Location</th>
<th>Hours of Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occupational Health Services</td>
<td>212-263-5020</td>
<td>One Park Avenue on the 3rd Floor</td>
<td>M-F 8:00AM-5:00PM</td>
</tr>
<tr>
<td>NYULMC Emergency Room</td>
<td>212-263-5550</td>
<td>560 First Avenue</td>
<td>Open 24 hours 7 days/week</td>
</tr>
<tr>
<td>Bellevue Hospital Emergency Department</td>
<td>212-562-5082</td>
<td>462 First Avenue</td>
<td>Open 24 hours 7 days/week</td>
</tr>
</tbody>
</table>

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

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

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

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

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

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

3. Reporting

Exposure incidents must be reported immediately either in person or by phone to a OCS Flow Cytometry Core Manager, the Co-Directors of the OCS Flow Cytometry Core, and OHS. Use Core Facility Spill/Accident Report Form, (Form FLOW-102F – a copy is at the end of this safety manual or can be obtained from the Laboratory Manager) to document the incident.
1. **Training Requirements**
   Personnel who want to ship or receive infectious substances must be current with training requirements.


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

2. **On-campus Transportation of Biological Samples**

   2.1. **Materials**

<table>
<thead>
<tr>
<th>Item</th>
<th>Manufacturer</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biohazard Bags</td>
<td>Lab Guard</td>
<td>19075388E</td>
</tr>
<tr>
<td>Hard container (cooler)</td>
<td>Igloo</td>
<td>7362</td>
</tr>
<tr>
<td>Blue absorbent pads</td>
<td>Fisherbrand</td>
<td>14-206-62</td>
</tr>
</tbody>
</table>

   2.2. **General Notes**
   - 2.2.1. All samples and containers must have biohazard labels.
   - 2.2.2. Avoid crowded areas whenever possible.
   - 2.2.3. The container should be carried directly to the intended laboratory - do not take the container to offices, cafeterias or other public or inappropriate locations.
   - 2.2.4. The package should be carefully inspected for signs of leakage or other contamination and, if necessary, decontaminated before opening.

   2.3. **Packaging Instructions**
   - 2.3.1. Label samples. Label information must include the identity of the biological material or agent, the universal biohazard symbol and the sending and receiving laboratory identification (e.g., Principal Investigator name and room number).
   - 2.3.2. Place sample in a primary container which is sealed and leak proof.
   - 2.3.3. Place the primary container in a secondary hard case container which is easy to decontaminate and capable of being securely closed.
   - 2.3.4. Liquid samples should be surrounded by enough absorbent pads in the secondary container to contain any liquids and absorb any shock during transport.
1. Materials

<table>
<thead>
<tr>
<th>Item</th>
<th>Manufacturer</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emergency flashlight</td>
<td>Sexauer</td>
<td>FST #456870</td>
</tr>
<tr>
<td>First Aid Kit</td>
<td>PhysiciansCare</td>
<td>Staples #503995</td>
</tr>
<tr>
<td>Smart Universal Power Supply 3000XL</td>
<td>ADP</td>
<td>-</td>
</tr>
</tbody>
</table>

2. Medical Emergencies

1.1. In case of a medical emergency, call the Medical Center's emergency number: 33-911.

1.2. If the individual is conscious and can be moved, remove him/her immediately out of the laboratories.

1.3. If the individual is unconscious and it will cause no further harm, the person will be immediately removed out of the laboratories and emergency personnel will be called to perform first aid.

1.4. If the victim cannot be moved, instruct the emergency responders of hazards and protective measures necessary in the facility.

1.5. Stay with the victim until emergency medical personnel arrive and take over.

2. Electrical Failures

2.1. In case of a power outage the operator must use his/her own best judgment to assess the situation and act accordingly.

2.2. In case of an electrical failure, call Bellevue’s main number for Facilities: (212) 562-4779.

2.3. Loss of power to the essential containment equipment will be avoided by use of a APC Smart UPS 3000xL battery backup system that will maintain power to the Aria Flow Cytometer, Whisper Aerosol Containment Unit, BioSafety Cabinet, and Vacuum pump for up to two hours, allowing proper shutdown and containment of biohazards.

2.4. If the blower fan of a BSC stops working any operator working in the BSC is required to cease all work immediately and exit from the laboratory following the exit procedures listed in SOP FLOW-101 for removal of protective gear.

2.4.1. The blower must be on for at least thirty minutes before work can resume.

2.5. In case of a blackout, all operators are to evacuate the facility. A rechargeable flashlight will be available for emergency use if needed.

2.6. Exit doors are identified with glow-in-the-dark exit signs which will allow the operator to find the exit door. Exit procedures listed in SOP FLOW-101 will be followed.

2.7. A sign should be posted on the entrance door with a notice advising persons not to enter the
facility.

3. Fire Emergency

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

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

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

3.2. After evacuating the facility on account of fire, all workers will remain at a safe distance to offer directions to the facility and any information EH&S and/or Fire Department personnel may request. When they or Fire Department personnel arrive on the scene, all workers will follow their instructions.
FORM FLOW-101F. BSL-2 Sorting User Approval

Name: _________________________________ Date: __________________

Date of birth: ______________

Principal Investigator: _____________________ Department: _______________

Title: _________________________________ Work phone: ______________

Home phone: ____________________________ E-mail: __________________

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

____ I have read (and received a copy of) the OCS Flow Cytometry Core’s SOPs and am familiar with:

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

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

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

User signature: _________________________________ Date: __________________

OCS Core Laboratory signature: ____________________ Date: __________________
Form FLOW - 102F. Core Facility Spill/Accident Report

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

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

1. Completed by (Name, Job Title):
   _______________________________________________________

2. Name/Job Title/Name of Principal Investigator: ______________________________

3. Date/Time of Incident: __________________________________________________

4. Infectious agent/hazardous substance involved: ______________________________________

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

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

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

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

9. What, if any, measures were taken to contain the safety problem?
   a. Evacuation of facility ____Yes  ____No
   b. Who de-contaminated the spill (person or persons)? ______________________________

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

11. List any injuries as a result of this incident:
    ____________________________________________________________________________
    ____________________________________________________________________________
12. What medical evaluation or treatment was sought due to the incident?

________________________________________________________________________________________
________________________________________________________________________________________
________________________________________________________________________________________

____

a. Emergency Room ____NYULMC _____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:
________________________________________________________________________________________
________________________________________________________________________________________
________________________________________________________________________________________
________________________________________________________________________________________
________________________________________________________________________________________
________________________________________________________________________________________
________________________________________________________________________________________
________________________________________________________________________________________