CYTOMETRY FACILITY
University of Pittsburgh Cancer Institute
Hillman Cancer Center- Research Pavilion
Suite 1.45, (412) 623-3282

Biosafety Level 2 plus (BSL-2+) Safety Manual

Flow Cytometry Cell Sorting Facility
UPCI Flow Cytometry Core Facility
Room 1.45, Hillman Cancer Center

May 2016

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University of Pittsburgh Cancer Institute
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Distribution: Flow Facility Staff, Investigators and staff using the MoFLo Astrios cell sorter or present during cell sorting.

Director Date
Albert D. Donnenberg, Ph.D.

Manager Date
E. Michael Meyer

University Biosafety Officer Date

Technologist and User Review

By signing I indicate that I have read and understand this manual and agree to abide by the policies set forth in this document. I also affirm that I have successfully completed and am current in the required modules on Bloodborne Pathogens and Chemical Hygiene.

Date	Signature

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1. Overview of Biosafety Level 2+ (BSL-2+) Facility & Procedures

Date: March 2016

Purpose: To provide a general overview of the facility and procedures for all personnel working in the BSL2+ laboratory of the Flow Cytometry Facility.

1.1 Introduction

The BSL-2+ facility involves moderate to high-risk agents and therefore requires a strict adherence to BSL-2 containment with BSL-3 work practices and procedures. It is important that all personnel that work in the BSL-2+ facility understand and adhere to the proper procedures and techniques outlined in this manual. Failure to adhere to appropriate practices and procedures may endanger others.

1.1.1 Information about agent in use.

Unfixed human cells from blood, body fluid, or other tissues are used in the facility. All untested human samples should be considered potentially infectious for HIV, hepatitis viruses and other bloodborne pathogens, which can infect humans through exposure of mucosal membranes to aerosol, broken skin or aerosol inhalation.

Retroviral and lentiviral transfected cells are used in this facility and are prepared and handled according to University Guidelines (Appendix II). The retroviral and lentiviral vectors are replication-defective, however, they will be inserted with some genes that may be oncogenic.

Cells potentially infected with the agents described above will be run through a high-speed cell sorter (MoFlo Astrios), which operates under high pressure (30-60 psi) and is capable of generating aerosols under normal operating conditions, especially when samples are sorted onto microscope slides. Although the fluidics of the Astrios are contained within a Class II safety cabinet, we require all personnel to observe universal precautions when handling live human cells.

Since hepatitis B virus is a bloodborne pathogen, immunization is strongly recommended.

1.2 BSL-2+ personnel requirements

All individuals working in the BSL-2+ facility must be trained according to the compliance policies of the University of Pittsburgh. Personnel receive annual updates, or additional training as necessary for procedural or policy changes. Additionally, the facility Director or Supervisor will conduct training for all personnel in the area, covering the potential hazards associated with the work, the necessary precautions to prevent exposures, exposure evaluation procedures, and the standard operating procedures of a BSL-2+ laboratory. Facility Director and

Supervisor are responsible for training employees concerning the hazards of the agents they will be working with and the proper laboratory techniques to use to avoid injury and illness. New employees must be trained prior to assignment in the lab. This training is to be documented in the employee's file with a listing of the session agenda, the name of the person providing the training, and the date and signature of the person trained. This record should be retained for the duration of employment and at least 3 years after.

Specialized training is required for the person operating the Astrios high-speed cell sorter. This training will be provided by the manufacturer of the Astrios or by cytometrists with extensive cell sorting experience. Training is to be determined by the Facility Director.

1.3 BSL-2+ facility, layout, and air handling

BSL2+ facility is located on the first floor of the Hillman Cancer Center, room 1.45 Annex. There are currently two instruments inside the lab: the MoFlo Astrios high-speed cell sorter (Astrios) and a Cellomics ArrayScan. There is one working bench and an additional Class II Biosafety Cabinet for the lab staff. There is one entry door to the Annex and a door to the adjoining main Cytometry Facility Lab.

Doors will be locked during sorting of unfixed, unknown human and non-human primate cells or lentivirus-transfected cells, as well as after working hours and on weekend.

The airflow in the laboratory is regulated centrally by Facilities Management to maintain a specified number of air changes per hour. The room air pressure is negative with respect to the corridor. The Class II Biosafety Cabinet in which the Astrios is housed exhausts into the room through a HEPA filter.

1.4 Medical requirements, hygiene, and good lab practices

1.4.1 Medical Requirements

All individuals present in the laboratory during the operation of the Astrios must meet the following medical requirements:

- Those with exposure to animals and/or their body fluids, fresh tissues, bedding, or caging are required by the University to enroll in the Animal Exposure Surveillance Program. Initial enrollment can be completed by downloading the enrollment form (available athttp://www.ehs.pitt.edu/assets/docs/AESPenroll.pdf) and faxing it to 412-647-1993. An update form is required to be completed every three years (available athttp://www.ehs.pitt.edu/assets/docs/AESPUpdate.pdf).
- All faculty and staff with exposure to human bloodborne pathogens are required to complete the following:
    - Those initiating work with materials potentially containing bloodborne pathogens are required to enroll in the University of Pittsburgh

Bloodborne Pathogen Exposure Control Program upon hire, and annual completion of BBP training.

- Serum Surveillance Program participation is optional through Employee Health Services (647-3407)
- Hepatitis B vaccination is strongly recommended and available without charge to individuals enrolled in the Bloodborne Pathogen Program.

1.4.2 Basic hygiene

It is mandatory that all personnel wear personal protective equipment (lab coats and gloves) when handling human specimens or working on the instrument. When cleaning the inside of a Class II biosafety cabinet, a face protection shield or goggles and a surgical mask and double gloves must be worn. All personnel must wash their hands after removing gloves. Eating, drinking, storing food, handling contacts, and applying cosmetics are not permitted in the laboratory. Food must not be stored in refrigerators or freezers in the laboratory.

1.4.3 General good laboratory practices

All unfixed specimens to be run on the Astrios cell sorter should be handled in a Class II safety hood using universal precautions. Mandatory laboratory practices include use of mechanical pipetting, use of plastic instead of glass, minimizing the use of sharps (see Section 3.1.8 for Sharps policy), labeling equipment with appropriate biohazard stickers, and minimizing work with infectious substances on the open bench.

2. Location of storage and use of BSL-2+ agents

Purpose: To provide a list of all agents used in the BSL-2+ lab and their storage and use locations:

Agents used in the BSL-2+ lab

- Unfixed or fixed human cells from blood, body fluid or other tissues
- Unfixed or fixed non-human primate cells from blood, body fluid or other tissues
- Retroviral or lentiviral transfected cells
- Cells from animal tissues, e.g. mouse, rat
- Human or animal cell lines from variety sources
- Beads for instrument alignment or QC
- Murine or rat derived monoclonal antibodies
- DNA stains (DAPI, PI, Hoechst 33342, DRAQ5)
- Formaldehyde (10% EM grade)

Location of storage of agents

Beads, monoclonal antibodies and DNA stains are stored in the refrigerator (4^0C) in the main laboratory. Formaldehyde is held in small quantities (< 1 liter) on the reagent shelf in the main laboratory. Generally, all the cells listed above are used immediately when they are brought to the lab. If the cells cannot be used immediately for some reason, they will be temporarily stored in the refrigerator in the main laboratory.

All reagents are eventually used on the Astrios high-speed cell sorter or analytical cytometers. After running unfixed, unknown human and non-human primate cells or lentivirus-transfected cells on the Astrios or on the analytical cytometers in the main laboratory, sample lines must be flushed with 10% bleach for at least 5 minutes followed by at least 5 minutes with DI water.

3. Standard Operating Procedures for the BSL-2+ facility

Purpose: To provide safe handling procedures and operations for personnel working in the BSL-2+ lab.

3.1 General BSL-2+ laboratory practices

3.1.1 Facility entry.

Access is restricted to those persons whose presence is required for experimental or support purposes. The entry door is locked when sorting is in progress and during non-working hours. The Facility Director and Supervisor have the final responsibility of assessing each circumstance and deciding who may enter or work in the laboratory. Only the door from the Main Laboratory to the Annex will be used to enter and exit the laboratory, except as noted in 1.3. The door to the main corridor will remain locked during sorting.

3.1.1.a Visitors.

Other individuals permitted to enter the laboratory when sorting is not in progress include personnel from Housekeeping who remove waste and clean the laboratory, and from Facilities Management and Information Services Division (ISD). Other visitors to the laboratory must be pre-approved by the Facility Director or Supervisor. Only approved laboratory workers may enter the laboratory during sorting of unfixed, unknown human and non-human primate cells or lentivirus-transfected cells.

3.1.1.b Contractors and vendors.

Field service engineers from Beckman Coulter and ThermoFisher, technical supporting personnel from the local maintenance department, and Filtech, Inc. are allowed to enter the lab for service purposes, as requested by lab staff, except when unfixed, unknown human and non-human primate cells or lentivirus-transfected cells are being sorted.

3.1.1.c Emergency medical personnel.

Emergency medical personnel may enter in the lab if they are notified that there is a medical emergency inside the facility. Efforts to secure all biological agents prior to emergency medical personnel entering the laboratory will be made.

3.1.2 Training requirements.

All personnel must complete the following training prior to working in the BSL-2+ laboratory. Training includes two Environmental Health and Safety training sessions (Bloodborne Pathogens and Chemical Hygiene training). These training sessions are held bimonthly. Bloodborne Pathogen training is required on an annual basis and Chemical Hygiene training is required every 3 years.

A special training will be required for the operators of the Astrios high-speed cell sorter. This special training will be provided by the manufacturer of the Astrios or by cytometrists with extensive cell sorting experience.

Workers cannot work in the BSL-2+ lab unless all the training requirements have been met, they have read and signed off on the manual, and received approval from the Facility Director and Supervisor.

3.1.3 Personal protective equipment.

Personal protective equipment (PPE) is designed to protect the worker from contact with biohazardous agents as well as to protect the work from contamination by the worker. PPE is considered a secondary line of defense against the infection. The primary line of defense is the use of Universal Precautions and good laboratory techniques. Mandatory PPE includes disposable lab coats or cloth lab coat and disposable sleeves and double gloves (latex over nitrile or nitrile over nitrile. When cleaning inside the hood, face protection (safety goggles or face shield and surgical mask) must be worn.

3.1.4 Biosafety cabinet (BSC).

The basic function of the biosafety cabinet (BSC) is to protect the user from exposure to infectious material, to protect the sample from potential contamination, and to prevent release of infectious materials to the environment.

The Class II Type BSCs in the sorting Annex must be certified annually by a qualified third-party vendor. All BSCs must be recertified after repair or relocation. The exhaust from this BSC Class II cabinet is HEPA filtered and is not connected to an external exhaust. It is connected to emergency power and will continue to operate in case of a power failure.

The BSCs are to be turned on at least five minutes before initiating work and will remain on at least 5 minutes after completion of work.

Normal operation of the Astrios-dedicated BSC is verified via the digital airflow gauge (≥0.28 in.w.c.) and alarm. The sorter will not generate a sort-stream if the BSC has not reached this preset value. After sorting the interior walls and work surface of the cabinet are disinfected with a 1:64 dilution of Vesphene IIse (Steris).

For the sample prep BSC, clean supplies are loaded as needed. Supplies should be wiped with a disinfectant to avoid introducing contaminants into the BSC work area. Limit arm movement in and out of the BSC to minimize disturbance of the air curtain, and keep the front and rear grilles free of obstructions to ensure proper function of the cabinet. All work in the sample prep BSC should be at least four inches behind the front grille. Ideally, one side of the cabinet is designated as the "clean" side, and the other side is designated as the "dirty" side. Personnel should work from "clean" to "dirty" to minimize contamination.

A waste container, such as a small biohazard bag or pipette tray, should be placed inside the cabinet, so that personnel do not have to remove wastes from the cabinet frequently, which can disturb the air curtain. Collect all solid wastes such as contaminated plastic ware in a bag in the biosafety cabinet and then dispose of it in a larger biohazard bag outside of the cabinet when work is completed. Personnel not working in the BSC should stay clear of the BSC to avoid disturbance of the air curtain. At no point should personnel place their faces or heads inside of the BSC. If outer gloves become contaminated during a work session, do not take your hands out of the BSC. Remove your contaminated outer gloves while your hands are still inside the BSC and dispose of the gloves in the waste container inside the BSC. Then remove your hands from the BSC and put on a fresh pair of outer gloves. At the end of the work session, all supplies should be disinfected before removal from the BSC. Wipe the exterior of tubes, plates, bottles, and/or small equipment (pipet aids, etc.). Decontaminate the interior walls with Versphen IIse as described above. Paper towels, gloves, and other materials used to decontaminate the BSC should be disposed of in biohazardous waste containers.

3.1.4.a.1 The Astrios dedicated Class II biosafety cabinet is provided with an interlock such that the Astrios can only be operated when the cabinet is working properly. An alarm will sound, a warning will appear on the cytometer control panel, and the sample stream will automatically be terminated if the air supply pressure is out of range. Additionally, the sorter itself is equipped with a dedicated aerosol evacuation system that is integrated into the sort chamber and vents into the BSC. This system is always active when the BSC is in operation.

3.1.4.a.2 The operator must uncap or recap samples inside the Astrios BSC.

3.1.4.a.3 1:64 dilution of Vesphene IIse phenolic disinfectant (Steris, Mentor OH) is used as disinfectant for the surface of biosafety cabinets and

the surface of the MoFlo instrument. Vesphene IIse solution is typically wiped or sprayed onto a surface and allowed to air dry. After Vesphene usage, an alcohol (70% EtOH) rinse of the surfaces should be completed followed by a sterile water rinse to prevent corrosion of the stainless steel surfaces. Note that EtOH alone is not sufficient for disinfection.

3.1.5 Decontamination of biological waste.

3.1.5.a Liquid waste.

All liquid wastes generated during BSL-2+ experiments should be immediately decontaminated by mixing with household bleach (6% sodium hypochlorite, final dilution at least 1:10 dilution but not greater than 1:50) for at least 30 minutes contact time. The solution may then be disposed of in the sink; however, the sink must be washed and decontaminated after.

3.1.5.b Aspiration of liquid waste. No vacuum utility is available in the BSL2+ sorting facility.

3.1.5.c Solid waste.

Solid wastes generated during sorting unfixed unknown human and non-human primate cells or lentivirus-transfected cells (e.g. pipettes tips, tubes) will be deposited into a beaker containing 1:10 dilution of household bleach to be located within the Astrios BSC. Waste materials must soak in bleach for at least 15 minutes before removing from the hood. Bleach-decontaminated waste will be strained in the sink. Decontaminated waste and used gloves will be disposed in a biohazard bag in a labeled cardboard container. 2/3 Full containers are sealed and removed by Housekeeping for autoclaving.

3.1.5.d Autoclave. Wastes disposed of in red biohazard bags are routinely autoclaved prior to disposal.

3.1.6 Transport of Agents.

Potentially infectious material transported into or out of the facility must be placed in a closed, leak-proof secondary container labeled with a biohazard sticker.

3.1.7 Centrifugation of Agents.

There is one centrifuge in the BSL-2+ sorting laboratory. Prior to use, make sure that all samples are properly placed inside the centrifuge with balanced carriers and carrier safety caps. The centrifuge is to be decontaminated with Vesphene IIse at the end of the day (on days that it is used). EH&S requires loading the centrifuge carriers/cups inside of the biological safety cabinet. After

centrifuge cycle is completed, the carrier/cups should be opened in a biological safety cabinet.

3.1.8 Sharps Policy.

The use of needles in the BSL2+ sorting laboratory should be minimized. Glass-slides will be used for the Astrios set up and sorting. Broken glass slides must be disposed of into a designated and labeled Sharps container. If Sharps (needles, scalpels, etc.) must be used in the BSL-2+ sorting laboratory, University Guidelines require the use of Safety-Engineered Sharps. EH&S should be contacted to provide information and trial devices if Sharps are required in the laboratory.

3.1.9 Exit Procedures.

Before leaving the work area, all solid and liquid wastes are to be disposed of in the proper manner (see 3.1.5 a and c). All equipment exposed to potentially biohazardous materials will be disinfected and returned to their correct place in the lab. Laboratory coats and gloves must be removed and disposed of in the biohazard solid waste container before leaving the lab.

3.2 Equipment maintenance.

3.2.1 Biosafety cabinets.

BSCs must be cleaned after each use. The surface of the BSC is wiped down with a 1:64 diluted Vesphene IIse solution. Filtech, Inc will certify BSCs once a year.

3.2.2 Centrifuges.

The centrifuge is to be cleaned and disinfected with Vesphene IIse at the end of the day (on days that it is in use).

3.2.3 Incubators. There are no incubators in the BSL2+ sorting facility.

3.3 Spill response in the BSL-2+ lab.

Spill control procedures are posted near the exits of the lab and Annex.

Spills of biological materials are decontaminated, contained, and cleaned up by appropriate professional staff, or others properly trained and equipped to work with concentrated infectious or potentially infectious material. Spills and accidents that result in overt exposures to BSL-2+ materials are immediately reported to the Facility Directors. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are kept.

<u>Biological Spills inside the BSC</u>

- Remove contaminated outer gloves, discard in the biohazard bag in the BSC, and remove hands from BSC. Disinfect and discard any other personal protective equipment (PPE) that may have become contaminated.
- Close the sash and allow the cabinet to operate for at least 5 minutes before proceeding with the spill cleanup.

- Notify others in the lab that a spill has occurred in the BSC.
- If any material has been splashed onto you, follow the procedure for Reporting Exposure to Potentially Infectious Material found in the University Safety Manual (EH&S Guideline #05-005). See Appendix A.
- Don clean PPE.
- Cover the spill with paper towels [or other absorbent material] to prevent further aerosol formation.
- Pour [approved disinfectant] gently over the covered spill, working from the outside inwards.
- Wait at least 15 minutes for the disinfectant to penetrate through the contained spill and achieve the required contact time for disinfection.
- Wipe up the spill working inward to the center of the spill. Avoid excess spraying of disinfectant as this can create more splashes and aerosols. Change gloves as needed. Do not use hands if glass or other sharps are involved in the spill. Use a tool (e.g. shovel or forceps) to remove the absorbent material and debris.
- Place all materials in a biohazard bag and repeat application of disinfectant. Allow for the appropriate contact time.
- Wipe off contaminated reusable supplies. Discard disposable contaminated supplies into the biohazardous waste.
- If the material spilled into the front or rear grille, lift the grille and disinfect both it and the waste basin underneath.
- Notify supervisor or PI.

<u>Biological Spills Outside of the BSC.</u> A major biological spill involves the release of BSL-2 or higher materials outside of a biological safety cabinet or involves excessive splashing or aerosol formation and requires assistance of EH&S and/or external emergency personnel.

- Alert personnel in the laboratory of the spill and direct additional personnel away from the spill area.
- If any material has been splashed onto you, follow the procedure for Reporting Exposure to Potentially Infectious Material found in the University Safety Manual (EH&S Guideline #05-005, Appendix A).
- Remove and disinfect any contaminated clothing.
- Notify supervisor, PI, and the Department of Environmental Health and Safety (EH&S) at 412-624-9505 of the incident.
- If the situation involves an imminently life-threatening injury, a release outside the building, or has other catastrophic potential, call 412-624-2121.

- Personnel knowledgeable of incident and laboratory should be available to assist EH&S and/or emergency personnel.

Contamination of the Astrios with a Known Pathogen In the event of contamination of the Astrios with a known pathogen due to a nozzle-clog during sorting resulting in the creation of an aerosol, the sort will be aborted and the Astrios and BSC will be decontaminated using vaporized hydrogen peroxide. EH&S operates two VHP units, which are reserved by contacting the University Biosafety Officer at 412-624-8919. The procedure for VHP decontamination was developed by EH&S and is included in Appendix 3.

3.6 References.

1. "Biosafety in Microbiological and Biomedical Laboratories – 4th Edition", CDC/NIH, U.S. Government Printing Office, Washington, D.C., 1999.
2. University of Pittsburgh Biosafety Manual.
3. Holmes, K. L., Fontes, B., Hogarth, P., Konz, R., Monard, S., Pletcher, C. H., Wadley, R. B., Schmid, I., and Perfetto, S. P. (2014) International Society for the Advancement of Cytometry cell sorter biosafety standards. Cytometry Part A 85, 434-453.

4. Emergency Response Guidelines

Purpose: To provide safe procedures for handling medical and facility emergencies in the BSL-2+ facility.

4.1 Emergency contact information. In the event of an emergency, the following contact information is posted near the phone in the BSL2+ facility.

Title, name	Phone number
Sorting Facility Director	**623-3256 or 623-7780**
Lab Supervisor	**623-3282**
Medical Emergency	**623-3131**
UPMC Presbyterian Emergency Department	**9-412-647-3333**
FIRE	**623-3131**
Chemical Spill	**623-2990**
Biological Spill	**9-412-624-9505**
Environmental Health and Safety (Pitt)	**9-412-624-9505**
EH&S Biosafety Officer	**9-412-624-8919**
University Police	**9-412-624-2121**
Employee Health	**9-412-647-3695**

4.2 Medical emergencies.

In the case of medical emergency, call 911 for help and file any on-the job injuries reports as required.

4.3 Injuries.

4.3.1 **On-the-job injury, bloodborne pathogen injury or sharps injury.** Report for treatment at:

Minor Injuries or Medical Surveillance

During normal working hours (7 am – 3:30 pm Mon-Fri)

Employee Health Services/UPMC Work Partners

In Shadyside: Aiken Medical Building, Suite 209

412-623-1920

Medical Emergencies at any Time or Minor Injuries After 3:30 pm or on Saturday / Sunday or holidays

Shadyside Hospital Emergency Department

412-623-2063

Minor Injuries or Medical Surveillance

During normal working hours (7:30 am – 4:00 pm Mon-Fri)

Employee Health Services/UPMC Work Partners

In Oakland: Medical Arts Building, Fifth Floor
412-647-3695
Medical Emergencies at any Time or Minor Injuries After 4:00 pm or on Saturday / Sunday or holidays

Presbyterian University Hospital Emergency Department
911 or 647-3333

Worker's compensation information. Work-related injuries should be reported to the University's Workers' Compensation department within one business day of an accident to preserve your right to benefits.

Employees that have been injured as a result of performing their work duties must call **1-800-633-1197** to report the injury (phone answers 24/7).

4.4 Facility emergencies.

4.5.1 Electrical failures. In case of a power outage, the back-up generators of the building will turn on, if not, find the emergency flashlight. If it happens during the sorting, stop sorting.

4.5.2 Exhaust failures. In case of an exhaust failure or change in negative air pressure, stop the ongoing jobs, secure open containers of biological agents, and leave the facility immediately and close the door behind you. Call for assistance. The laboratory should be signed "Do Not Enter – Emergency Personnel Only"

4.5.3 Fire emergencies. In case of a fire, walk to the nearest exit of the facility and go to the nearest staircase. Walk down and exit the building.

5. Revision History

Revision	Change	Rationale	Standards	Start Date	End Date
0	Creation	Commissioning of CytoShield	29 CFR 1910.1030	11/02/04	12/07/04
1	Final recommendations of EHS	Implementation	n/a	12/07/04	1/19/04
2	Update of instruments, lab organization				
3	Environmental Health and Safety edits (Mark DiNardo)	Update of EH&S information	n/a	1/20/04	12/10/09
4	Deletion of SortMaster related procedures, Changes to SOP for operation of the MoFlo.	SmartSampler allows for remote operation. Sorting permissible on samples containing BSL2 pathogens as determined in a case by case basis. Addition of "Decontamination after sorting."	n/a	12/11/09	3/27/16
5	Update for BSC II-contained Astrios	New procedure relating to change from BSC I to BSC II containment and move of sorter to the isolated laboratory Annex	ISAC 2014 recommendations for cell sorting (Appendix 4)	Not implemented	
6	EH&S recommendations	Deleted VHP decontamination, updated standard operating procedure specific to MoFlo Astrios	n/a	5/31/16	

6. Appendices

1. SOP for operation of the Astrios high-speed cell sorter
2. Research with Lentiviruses and Lentiviral Vector Systems, University of Pittsburgh Safety Manual, 12/1/2014.
3. International Society for the Advancement of Cytometry Cell Sorter Biosafety Standards. Holmes, KL et al. Cytometry A 85A: 434, 2014
4. Bloodborne Pathogen Accident Treatment and Reporting

Appendix I

SOP for MoFlo Astrios high-speed cell sorter at University of Pittsburgh Cancer Institute

Introduction

The MoFlo Astrios high-speed cell sorter manufactured by Beckman Coulter is a jet-in air cell sorter. It operates under relative high pressure (60 psi) and is capable of generating generate aerosols under normal operating condition. The risk of a high-pressure aerosol is greatly increased when the sorter's nozzle tip becomes obstructed. The Astrios high-speed cell sorter at University of Pittsburgh Cancer Institute operates in a Class II biosafety cabinet and is designed to contain aerosols generated in the sorter. In order to sort unfixed unknown human and non-human primate cells or lentivirally-transfected cells, the Astrios is located inside a dedicated BSL2+ laboratory. This SOP is designed to provide an operational guideline for sorting potentially biohazardous samples in the BSL2+ environment.

Personal requirements for operators

- Proper training for instrument operation
- Training in Bloodborne Pathogens, Chemical Hygiene, and participation in the Animal Exposure Surveillance Program (mandatory for animal workers)
- Participation in the Serum surveillance program (optional)
- Hepatitis B virus immunization (optional, strongly recommended).

Laboratory practice

- No food and drink allowed in the lab
- Limit unnecessary entry to the lab during the sorting
- Lock the door during the sorting
- A sign of " sorting in progress" on the door during the sorting
- A disposable lab coat is required for all the persons involved in sorting
- Double Gloves are required for all the persons handling sorting samples

Sample acceptance criteria

- Samples known to contain BSL3 pathogens are not acceptable for sorting. Some BSL2 pathogens (considered as BSL3 in practice as described in Holmes, K. L. et al, (2014) International Society for the Advancement of Cytometry cell sorter biosafety standards. Cytometry Part A 85, 434-453) require case by case evaluation by the Facility Director in consultation with the Facility Supervisor and the Biosafety Officer. See Appendix III.
- Sample must be contained in a leak proof container and clearly labeled with a sample identifier and a biohazard symbol.

- Sample must be clump free and in an appropriate tube for sorting (12 x 75 mm snap cap tube)
- Sample must not contaminate the outside of the tube

Sample handling

- The operator and other individuals handling the specimen must wear liquid barrier double gloves.
- All individuals present during a sort must wear a disposable lab coat.
- If the sample requires transfer or filtration, this must be done in the BSC2 using universal precautions
- The sample must be uncapped inside the BSC by operator.
- The sample must be recapped inside the BSC prior to removal
- The sorted cells must be capped or covered inside the BSC prior to removal.
- Unused cell samples and sorted cells must be placed in caped, labeled tubes. It is the responsibility of the laboratory that provided the sample to remove unused cell samples and sorted cells from the Flow Facility.

Routine procedure to start a sort

- Make sure that the BSC is on and functioning normally 5 minutes prior to turning on the Astrios. The Astrios will not generate a sort stream if the BSC is not operating at the proper pressure differential.
- Make sure the sheath tank is full.
- Empty the waste tank into the sink only after the waste liquid has been exposed to 1:10 dilution of bleach for at least 30 minutes. Pour concentrated bleach into the tank to make sure that there is about 1:10 dilution of bleach when the tank is full.
- Turn on the Astrios according to the manufacturer's instructions.
- Align the instrument before sorting.
- Make sure sample is free of clumps before acquisition. Filter if necessary in the BSC II next to the sorter.
- Set up the sample collection receptacle (*e.g.* tubes, plate, slide) in the sort-chamber.
- Close the sort-chamber door prior to initiating a sort.
- Operator should never leave the sample unattended while sorting unfixed unknown human and non-human primate cells or lentivirally-transfected cells.

The operator must terminate the sort if

- The operator detects a clog or stream irregularity on the video monitor.
- The system will shut down automatically if the BSC fails. If this occurs, close the front sash on the BSC and let any potential aerosol settle for at least 2 hours before initiating decontamination.

Routine procedure to stop a sort in the event of a clog or stream irregularity

- Terminate sort
- Don liquid barrier double gloves and open sort chamber door
- Wait 2 minutes before removing sample
- Wipe sample tube with 1:10 dilution of bleach or 70% EtOH (only if BSL-1).
- Wash sample line by flushing for at least 5 minutes with a 1:10dilution of bleach stock in DI water, followed by at least 5 minutes with DI water at > 1.0 sample pressure differential.
- Disinfect inside surface of the BSC and the sort-chamber with 1:64 diluted Vesphene IIse.
- Wear face shield or goggle and surgical mask when cleaning inside of the BSC
- Replace the collection tubes
- Continue with sort

Routine Maintenance

- Daily routine clean the surface of inside biosafety cabinets by using 1:64 diluted Vesphene IIse
- Check sheath line and waste line daily for wet due to leaking. Replace any leaking lines immediately
- Rinse sheath tank with clean sheath if there is debris or crystals inside the tank
- Certify BSC annually according to NSF/ANSI Standard 49 (2012).

References

1. CDC. Recommendations for prevention of HIV transmission in health-care settings. MMWR 1987;36 (suppl no. 2S).
2. CDC. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. MMWR 1988;37:377-388.
3. CDC. Guidelines for prevention of transmission of human immunodeficiency virus and hepatitis B virus to health-care and public-safety workers. MMWR 1989;38(S-6):1-36.
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Appendix II

University of Pittsburgh Safety Manual	EH&S Guideline Number: **05-016**	
Subject: **Lentiviruses and Lentiviral Vector Systems**	Effective Date 12/1/2014	Page 1 of 7

RESEARCH WITH LENTIVIRUSES AND LENTIVIRAL VECTOR SYSTEMS

1. SCOPE

This guideline describes biosafety considerations and work practices for work with human pathogenic lentiviruses and lentiviral vector systems at the University of Pittsburgh.

2. DEFINITIONS

2.1 Lentivirus(es): Lentiviruses are a subset of retroviruses that have the ability to integrate into host chromosomes and to infect non-dividing cells, and include human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) which can infect humans. Other commonly used lentiviruses that are infectious to animals, but not humans, include feline immunodeficiency virus (FIV) and equine infectious anemia virus (EIAV).

2.2 Lentiviral Vectors: Lentiviral vectors consist of recombinant or synthetic nucleic acid sequences and HIV or other lentivirus-based viral packaging and regulatory sequences flanked by either wild-type or chimeric long terminal repeat (LTR) regions.

2.2.1 Replication-Deficient Lentiviral Vectors: Certain lentiviral vectors are designed to be less pathogenic than wild-type lentiviruses due in part to the separation of genes required for packaging of viral particles onto several plasmids, replacement of the native lentiviral envelope protein, and elimination of accessory genes that are essential for replication of wild-type lentiviruses. Lentiviral vector systems designed with these enhanced safety features are not able to replicate in human cells and are defined as replication-deficient lentiviral vectors.

2.3 Employees Potentially at Risk: Laboratory workers handling pathogenic lentiviruses, recombinant lentiviral vectors, naturally or experimentally infected laboratory animals, or clinical specimens potentially infected with lentiviruses are at risk.

2.4 Laboratory Hazards: Penetration through the skin via puncture, cut, or absorption through broken skin (*e.g.* scratches, cuts, abrasions, dermatitis, or other lesions) and/or mucous membrane exposure via splash to the eyes, nose, and/or mouth are known to be

potential exposure pathways for lentiviral agents.

3. REGISTRATION REQUIREMENTS:

All Principal Investigators (PIs) working with clinical specimens containing blood, blood components, body fluids and/or tissues from humans, cultures of lentiviruses or lentiviral vectors, or animals or unfixed specimens from animals infected with lentiviruses or lentiviral vectors must be registered with Environmental Health & Safety (EH&S).

3.1 Use in Animals: Registration is accomplished via completion of an electronic IACUC protocol for work with animals involving lentiviruses or lentiviral vectors.

3.2 Use *in vitro* and/or Use of Recombinant or Synthetic Nucleic Acids: Investigators performing solely *in vitro* experiments, should complete a registration document obtained from www.ehs.pitt.edu/biological/workbook.html. PIs working with recombinant or synthetic nucleic acids in lentiviral vectors or performing recombinant or synthetic techniques using genetic material from lentiviruses must be registered with the Institutional Biosafety Committee. IBC application documents may be obtained from http://www.ibc.pitt.edu/Apply/apply.htm.

4. ASSIGNING BIOSAFETY LEVELS FOR WORK WITH LENTIVIRUSES

4.1 Biosafety Level Assignment Work Practice Guidance:

The following guidelines are provided to assist in the determination of the appropriate biosafety level and work practices to be used with lentiviruses and lentiviral vectors. For work with lentiviruses, biosafety level assignment is based on the research-specific risks (*e.g.* sample population, specific hazards associated with techniques), pathogenicity of the lentivirus in use, and design of laboratory facilities. **Final biosafety level determination for work with lentiviruses and lentiviral vectors containing recombinant or synthetic nucleic acids will be made by the Institutional Biosafety (IBC) Committee.** The University Biohazards Committee will provide guidance as necessary, and must be consulted for all work being considered at the BSL-3 level.

4.1.1 Biosafety Level 2 (BSL-2): BSL-2 is appropriate for:

4.1.1.1 Work with diagnostic specimens that contain human blood, body fluids or tissues,

4.1.1.2 Generating and using IBC-approved, replication-deficient lentiviral vectors,

4.1.1.3 Work with lentiviruses or lentiviral vectors based on lentiviruses such as FIV and EIAV that are not known to be infectious to humans, and

4.1.1.4 Handling animals, animal tissues, blood, body fluids and/or cell lines infected with replication-deficient lentiviral vectors, that **do not** express oncogenes, if the vectors meet all other criteria listed in Table 1 below.

4.1.2 Biosafety Level 2+ (BSL-2+): At the University of Pittsburgh, there is a specialized biosafety level defined as BSL-2+. In BSL-2+ laboratories, additional work practices and containment equipment described in biosafety guidelines for work at BSL-3 are used in a BSL-2 facility (additional detail is provided below in Section 4.2). BSL-2+ is appropriate for:

4.1.2.1 Processes that include culture and production of known or potentially human pathogenic lentiviruses (*e.g.* HIV, SIV, recombinant forms of HIV/SIV/SHIV),

4.1.2.2 Manipulation of human pathogenic lentivirus-infected samples for research purposes,

4.1.2.3 Use of lentiviral vectors that contain oncogenes or that are not replication deficient (see Table 1),

4.1.2.4 Manipulation of high-titer virus preparations in volumes ≥ 100 mL but ≤ 10 L, and/or,

4.1.2.5 Procedures with a high likelihood of droplet or aerosol formation.

4.1.3 Biosafety Level 3 (BSL-3): Activities involving large scale preparation (≥ 10 Liters) of concentrated, high-titer lentiviruses shall be conducted in an approved BSL-3 facility, using BSL-3 practices and containment equipment. Any BSL-3 work must be approved by the University Biohazards Committee and EH&S. Additional approval from the IBC is required for work with large scale volumes of lentiviral vectors modified with recombinant and/or synthetic nucleic acids.

4.1.4 Animal Housing: Animals infected with lentiviruses and/or lentiviral vectors shall be housed in ABSL-2 facilities at a minimum. The IBC may assign work with animals to ABSL-2 or ABSL-3 depending on specific hazards and risks of the project, including but not limited to, animal species, specific agent, and experimental manipulations.

4.2 Definition of BSL-2+ at the University of Pittsburgh: Following completion of registration, laboratories are inspected by EH&S to verify appropriate containment and work practices. The criteria for BSL-2+ includes meeting all facility containment requirements for BSL-2, while following specific BSL-3 work practice requirements, as outlined in *CDC Biosafety in Microbiological and Biomedical Laboratories*, 5th edition, and the *NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules*, latest edition.

The specific additional practice requirements for BSL-2+, in addition to all standard work practices required at BSL-2, shall, at a minimum, include the following:

4.2.1 An investigator-specific BSL-2+ Biosafety Operations Manual must be prepared by the investigator, and must include:

4.2.1.1 An approval page signed by the PI and the Biosafety Officer, and the animal facility director if work with lentiviruses or lentiviral vectors involves animals,

4.2.1.2 Emergency contact numbers for laboratory management personnel,

4.2.1.3 Agents to be used, locations of use, and details regarding safe, access-controlled storage of agents,

4.2.1.4 Procedures describing proper response and clean-up of spills of lentiviral cultures, infected cell cultures, and other potentially infectious

material,

4.2.1.5 Procedures describing proper first aid and medical response procedures for personnel who may be exposed to lentiviral cultures , infected materials or animals,

4.2.1.6 Laboratory-specific training requirements for personnel who will work with lentivirus cultures, and/or infected cells and animals as well as records demonstrating completion of this training,

4.2.1.7 Laboratory-specific standard operating procedures for routine laboratory tasks, including safe handling of infectious agents, required facility-specific personal protective equipment (PPE), decontamination and disposal of waste, and

4.1.2.8 Autoclave verification program, if autoclave is used for decontamination.

4.2.2 Access to laboratory must be restricted to personnel trained in the contents of the Biosafety Operations Manual while work with lentiviruses or lentiviral vectors is in progress.

4.2.3 The laboratory shall be negatively pressurized to surrounding spaces.

4.2.4 The Principal Investigator is responsible for ensuring that all personnel *demonstrate proficiency* in the practices and operations of the facility prior to beginning unsupervised work at BSL-2+.

4.2.5 All vacuum lines used to aspirate infected cultures must be protected with liquid disinfectant traps and in-line HEPA filters (BMBL 5th Edition, Appendix A, Figure 12).

4.2.6 A certified Biological Safety Cabinet (BSC) *must* be used for all manipulations involving infectious materials.

4.2.7 Centrifuge safety cups and/or safety rotors *must* be used for centrifugation outside of a BSC. Safety cups and safety rotors must not be opened for loading/unloading of samples outside of a certified BSC.

4.2.8 Personal protective equipment must consist of the following and must be worn at all times within the BSL-2+ facility:

4.2.8.1 Either 1) a disposable, liquid-barrier wrap around gown, or 2) a standard, BSL- 2+ facility-dedicated button front lab coat with a liquid-barrier wrap around apron and disposable sleeve covers;

4.2.8.2 Mucous membrane splash protection consisting of a full face shield or safety glasses, in combination with a surgical mask for anticipated splashes or sprays of infectious materials.

4.2.8.3 Double gloves (latex over nitrile or nitrile over nitrile);

4.2.9 All personal protective equipment must be removed and either properly decontaminated and stored, or disposed of prior to leaving the

laboratory.

4.2.10 All potentially contaminated solids and/or liquids must be properly decontaminated prior to removal from the facility (*e.g.* soaked in bleach and disposed of in infectious waste stream; surface decontaminated with an appropriate EPA-registered disinfectant; double- bagged and autoclaved; liquids decontaminated with appropriate EPA-registered disinfectant prior to sink disposal)

4.2.11 Animal cages must be autoclaved before they are cleaned and washed.

4.2.12 Personnel must be notified that serum surveillance is available to all individuals potentially exposed to lentiviruses or lentiviral vectors. See EH&S Guideline 05-11: Serum Surveillance Program (http://www.ehs.pitt.edu/assets/docs/serum-surveillance.pdf).

5. ADDITIONAL GUIDANCE FOR ASSIGNING BIOSAFETY LEVELS FOR WORK WITH LENTIVIRAL VECTORS

5.1 Biosafety Level Assignment Guidance: Biosafety considerations are based on the specific lentivirus or lentiviral vector system used, the potential for oncogenic activity of expressed genes, and scale of production of the lentivirus or lentiviral vector. **Final biosafety level determination will be made by the Institutional Biosafety Committee.** The University Biohazards Committee must be consulted for any work being proposed for BSL-3/ABSL-3.

5.2 Expression of Oncogenes or Genes with Oncogenic Activity in Lentiviral Vectors: All lentiviral vectors expressing oncogenes or genes with oncogenic activity must be handled at BSL-2+, regardless of the packaging system used.

5.3 Production of Concentrated Lentiviral Preparations: Investigators planning to produce quantities of concentrated, high-titer lentiviral vectors by preparing 100 mL or more of culture, but not exceeding 10 L, must produce lentiviral preparations at BSL-2+ regardless of packaging system used.

5.4 3rd Generation, 4-Plasmid (or more) Lentiviral Systems: It is strongly recommended that investigators use 3rd generation, 4-plasmid lentiviral vector systems available from commercial vendors, recognized vector cores, or other investigators at the University of Pittsburgh. The IBC does not require testing for replication competent viruses (RCV) for generation and/or use of small volumes (≤ 100 mL) of 4-plasmid (3rd generation) systems that do not express genes with oncogenic activity at BSL-2/ABSL-2 (Table 1).

5.5 2nd Generation, 3-Plasmid (or less) Lentiviral Systems. 2nd generation, 3-plasmid lentivirus systems must be generated and used at BSL-2+. Investigators may request to conduct such research at BSL-2 following demonstration that virus preparations have no detectable RCV. If this data is not available at the time of original IBC protocol submission, an IBC protocol modification requesting BSL-2 conduct must be submitted. The modification must detail the type of testing used to ensure that no detectable RCV is produced and the data from the RCV testing if performed by the investigator (see section 4.6 below).

IBC approval of the modification must be received in writing before any 2nd generation, 3-plasmid lentiviral systems may be handled at BSL-2 (Table 1).

Approval for BSL-2 handling of 2nd generation, 3-plasmid systems at BSL-2 shall be specific to each virus preparation made. If separate preparations expressing different recombinant or synthetic nucleic acids are generated, each preparation must be demonstrated to be free of RCV and approved by the IBC prior to handling at BSL-2.

Table 1. Summary of biosafety level requirements for lentiviral vector production and use

Oncogenic transgene or >100 ml production	No. of plasmids	RCV testing	Vector production	Use of viral vectors in vitro	Use of viral vectors in animals	Use of virus-transfected cells in animals
Yes	Any number	Not required	BSL-2+	BSL-2+	ABSL-2+	ABSL-2+
No	4 or more	Not required	BSL-2	BSL-2	ABSL-2	ABSL-2
	3	Elect to test for RCV	BSL-2+	BSL-2 if approved by IBC	ABSL-2 if approved by IBC	ABSL-2 if approved by IBC
		No RCV test	BSL-2+	BSL-2+	ABSL-2+	ABSL-2+

5.6 Replication Competent Virus Assay: Testing for RCV may be performed by individual investigators using a standard p24 ELISA kit, provided that the assay has a sensitivity of $\leq$ 12.5 pg/ml.

A positive control for virus infection is not required; neither the IBC nor EH&S want the investigator to work with infectious HIV-1 for this assay. However, the assay must contain a positive control for the ELISA itself in the form of p24 antigen.

Virus should be tested for RCV by serial passage of tissue culture supernatant on 293T cells for 3 passages with subsequent testing of supernatant from each passage for p24 antigen by ELISA.

The Magee-Women's Research Institute Transgenic and Molecular Core (Director: Kyle Orwig; Administrator: Jennifer Orwig; phone 412 641-2415) is available for a fee to perform the RCV assay for individual investigators. If the MWRI Transgenic and Molecular Core performs the assay for the investigator, the p24 ELISA assay report provided by the Core must be submitted to the IBC office.

5.5 Exceptions to the requirement for RCV testing of 3-plasmid lentivirus stock. Investigators who acquire prepared lentiviral vector stocks from a commercial source that provides documentation of acceptable RCV testing will not be required to test for RCV. Manufacturer-specific RCV testing information must be included in the IBC Protocol application.

Investigators who are not generating their own viruses from a 3-plasmid system, but are acquiring prepared lentiviral vector stocks from a University of Pittsburgh investigator, an established University

Vector Core laboratory, or an investigator from another institution should contact the IBC regarding requirements for RCV testing, which will be reviewed on a case-by-case basis.

6. REFERENCES

6.1 Biosafety in Microbiological and Biomedical Laboratories. 5th edition. Washington, D.C. Centers for Disease Control and Prevention and National Institutes of Health; December, 2009.

6.2 NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. Bethesda. The National Institutes of Health Office of Biotechnology Activities; November, 2014.

6.3 Biosafety Considerations for Research with Lentiviral Vectors: Recombinant DNA Advisory Committee (RAC) Guidance Document. The National Institutes of Health Office of Biotechnology Activities. http://osp.od.nih.gov/sites/default/files/resources/Lenti_Containment_Guidance_0_0.pdf; Accessed 06/26/2014.

6.4 University of Pittsburgh Institutional Biosafety Committee Guidance on Biosafety Level Assignment for Lentiviral Vectors. University of Pittsburgh IBC. http://www.ibc.pitt.edu/ViralVector/ViralVector.htm; Accessed 06/26/2014.

Appendix III

ORIGINAL ARTICLE

Cytometry
PART A

International Society for the Advancement of Cytometry Cell Sorter Biosafety Standards

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Abstract

Flow cytometric cell sorting of biological specimens has become prevalent in basic and clinical research laboratories. These specimens may contain known or unknown infectious agents, necessitating precautions to protect instrument operators and the environment from biohazards arising from the use of sorters. To this end the International Society of Analytical Cytology (ISAC) was proactive in establishing biosafety guidelines in 1997 (Schmid et al., Cytometry 1997;28:99–117) and subsequently published revised biosafety standards for cell sorting of unfixed samples in 2007 (Schmid et al., Cytometry Part A J Int Soc Anal Cytol 2007;71A:414–437). Since their publication, these documents have become recognized worldwide as the standard of practice and safety precautions for laboratories performing cell sorting experiments. However, the field of cytometry has progressed since 2007, and the document requires an update. The new Standards provides guidance: (1) for laboratory design for cell sorter laboratories; (2) for the creation of laboratory or instrument specific Standard Operating Procedures (SOP); and (3) on procedures for the safe operation of cell sorters, including personal protective equipment (PPE) and validation of aerosol containment. Published © 2014 Wiley Periodicals Inc.†

Key terms

flow cytometry; occupational health; biohazards; cell sorting; biosafety; aerosol containment

Introduction

In 1994 the International Society of Analytical Cytology (renamed International Society for the Advancement of Cytometry in 2006; ISAC), an association representing researchers involved in cytometry, recognized the need to formulate safety guidelines for sorting and analysis of unfixed cells. ISAC initiated the formation of a Biohazard Working Group and the formation of a Biosafety committee, which published the official guidelines in 1997 (1). These guidelines provided recommendations for practices to reduce the potential for biohazard exposure of instrument operators. As stated in the preface to those guidelines, revisions were expected to occur at periodic intervals, and in 2007 the ISAC Biosafety Standards for sorting of unfixed cells was published (2). Although these standards reflect valid procedures and practices, an update is relevant at this time for the following reasons:

1. The ISAC Biosafety Committee disseminated an online survey to ISAC members that elicited responses on a variety of biosafety related topics. As a result of this survey, it became evident that certain areas in the current standards warranted clarification. For example, the appropriate biosafety procedures for sorting of human cell lines and of lentivirus-infected cells are often difficult to determine, hence leading to questionable sorting practices.
2. A more thorough characterization of aerosols capable of being produced by cell sorters has recently been published (3).

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Appendix IV

Blood-Borne Pathogen Accident Treatment and Reporting