Temple University, School of Medicine

Safety Standard Operating Procedure

BD Influx cell sorter in the Flow Cytometry Facility

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Date: 4/10/2010

1. **INTRODUCTION**

This procedure covers the processing of samples at biosafety levels 1 & 2 (BSL-1 & BSL-2). BSL-1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. Both containment levels are described in the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories.

The Influx cell sorter is a 'jet in air' type cell sorter that generates cell containing aerosols. Droplets larger than 80 μm constitute the majority of the droplets generated during sorting, however large amounts of secondary aerosols of various droplet sizes can occur during sort failures, for example, when the fluid stream splatters on a solid surface rather than into the trough of the waste catcher. When sorting BSL-2 materials, the droplet-based sorting procedures are considered BSL-3 practice (see table 1 from reference 1). Alternatively, containment systems that prevent aerosol escape by completely enclosing cell sorters into a Biocontainment Biological Safety Enclosure will permit to sort BSL-2 classified materials using BSL-2 practices (reference 1).

The Influx cell sorter in the flow cytometry facility is confined in the HEPA filtered enclosure and equipped with smoke evacuation system designed for personnel protection. This design allows to analyze and sort live human cells and other BSL-2 materials using enhanced BSL-2 practices. In addition to the annual certification of the HEPA filtered enclosure, the containment of the system is quickly checked by Glo-Germ method under sort failure mode before each sort of BSL-2 materials (reference 1). Only trained staff of the flow cytometry core facility can operate the BD Influx cell sorter.

This SOP does NOT cover samples requiring BSL-3 containment, which is appropriate for work with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. The safety of the flow cytometry facility staff and its users is our ultimate concern. To assure the assignment of proper safety practices and procedures, it is necessary to collect detailed information about all samples prior to receipt. A Hazard Assessment Form must be completed and submitted to staff of the flow cytometry core facility at least 48 hours prior to the each sort. Work will not proceed until safety approval is granted. Each investigator is also required to complete the Principal Investigator Form with current IBC, IAUCUV number.

2. BIOSAFETY LEVEL

At a minimum, all research laboratory work conducted is conducted in full accordance with BSL-2 requirements. This baseline containment is referred to as "Biomedical Research Lab" status and is communicated by a green doorway sign.

Human specimens and some other potentially infectious materials require BSL-2

containment with two primary enhancements: 1) open container work is confined to a HEPA filtered enclosure, and 2) required PPE includes seamless front gown, full–face protection, and gloves. This enhanced BSL-2 containment is referred to as “Human Specimen/Potential Infectious Materials” status and is communicated by a red doorway sign.

Fixed human cells or uninfected rodent samples
Cells that have been fixed by a proven method should not pose an infectious risk. Experimental rodent cells that have not been purposely infected with infectious microbes, should not pose a significant biohazard. Analysis and sorting of these cells can be performed observing “Biomedical Research Lab” status.

Unfixed human and unfixed non-human primate (NHP) samples
Many unfixed human or NHP cells have the potential to harbor infectious agents such as hepatitis viruses, human immunodeficiency virus, and other bloodborne pathogens. Such cells are assigned to BSL-2 containment while observing “Human Specimen/Potential Infectious Materials” status.

Recombinant DNA and Viral Vectors
The manipulation of rDNA materials must be approved by the IBC prior to performing the work. Each approved rDNA project is assigned a containment level by the IBC. Work with material assigned to BSL-1 or BSL-2 will be covered by this SOP. When enhanced BSL-2 is required, the work will be assigned to containment consistent with “Human Specimen/Potential Infectious Materials”. Additional requirements by the IBC will also be observed.

3. UNIVERSAL PRECAUTIONS

All unfixed human and NHP cell preparations shall be handled while observing universal precautions, the concept that such materials are treated as if known to be infectious for HIV, HBV and other bloodborne pathogens. All work with human material require:

- Annual bloodborne pathogens training
- Full conformance with the Exposure Control Plan. Complete ECP Personnel Sign in List.
- Compliance with the institutional hepatitis B vaccine policy. All employees who decline to accept hepatitis B vaccination are required to sign a Hepatitis B Vaccine Declination Form.

4. PERSONAL PROTECTIVE EQUIPMEN

“Biomedical Research Lab” status:

- Close-toed shoes
- Lab coat or gown
- Disposable gloves for handling samples
- Full-face protection when splash is likely

“Human Specimen/Potential Infectious Materials” status:

- Close-toed shoes
- Seamless-front gown with cuffs
- Double disposable gloves
- Full-face protection

5. TRANSPORT OF SAMPLES

- At a minimum, materials are double-packaged prior to transport. At least one layer of packaging must be leak-proof if the material is a liquid.
- All human samples must be transported in an approved secondary container. The Nalgene® BioTransport Carrier (Nalgene No. 7135) is recommended.
- The outer sides of the carrier must be sprayed with a bleach solution (1-10%) and wiped. Hard plastic exteriors are best for cleanability. Once this step is complete, the outer surfaces of the carrier are considered safe for handling with bare hands. Gloves should not be worn to transport carriers outside the laboratory.
- A biohazard warning label shall be applied to the carrier to communicate the potential hazard of its contents.

6. HAZARD SIGNAGE

A universal biohazard warning sign shall be posted at the doorway to the laboratory when potentially infectious materials are present. The sign will indicate the biosafety level, the types of hazards in the lab (e.g., unfixed human cells) and a contact name and phone number. In addition, the signage will communicate entry and exit requirements for the laboratory.

7. HANDWASHING

Hands must be washed as soon as possible when they come in contact with potentially infectious materials. A vigorous handwashing with a mild soap and water for 15 seconds is appropriate. Hands must be washed as soon as feasible after gloves are removed, and before exiting the laboratory.

8. DECONTAMINATION PROCEDURES

Spill procedure

- Alert people in the immediate area.
- Remove contaminated personal protective equipment.
- If eyes are exposed, flush them with the eyewash. Wash any potentially exposed skin with soap and water.
- Put on clean personal protective equipment.
- Clean area of all visible fluids with detergent (soap/water)
- Cover the spill with paper towels.
- Prepare a fresh 1:10 dilution of household bleach.
- Pour the 1:10 bleach around the edges of the spill, then onto the spill.

- Allow 20-minute contact time, then use the absorbent material to wipe up the spill, working from the outer edges to the center.
- Clean the spill area again with fresh absorbent material soaked with 1:10 bleach.
- Discard these materials in an autoclave bag for decontamination by autoclaving.

Biowaste Decontamination

Solid waste

- Collect in a biohazard bag.
- Loosely close the bag by taping with temperature indicator tape.
- Spray the exterior of the bags with disinfectant.
- Place into the waste transport container.
- Autoclave for 90 minutes at 121°C.

Pipettes and sharps

- Place in puncture-proof containers prior to placement into biohazard bags.

Liquid waste

- Carefully add straight bleach to the solution without splash. Approximately 10% of the total solution will be made up of bleach.
- Cap the container and gently swirl to ensure mixing.
- Allow 30 minute contact time and then carefully pour down the sink.

Cell Sorter Decontamination

At the end of each experiment or prior to service work on the sorter, the unit is decontaminated by the following procedure:

- Place sort tube holders in a 10% bleach solution for 30 min, spray and wipe with 70% ethanol.
- Spray and wipe surfaces inside the sort chamber with 10% bleach followed by 70% ethanol.
- Run 10% bleach 10 min, 70% ethanol, then deionized water through the sample tubing.

9. MEASUREMENT OF CONTAINMENT AND TOLERANCES

- The containment is tested under sort failure mode. The sorter is set to use 70 μm nozzle and 70 psi, with the waste catcher blocked to create large aerosol.
- Turn smoke evacuation system and HEPA filtered enclosure on. Close door to the sort chamber.
- Add glass slide to the AeroTech concentrator. Apply vacuum to the AeroTech concentrator.
- Run Glo-Germ particles (5-mm melamine copolymer resin beads in a 5-ml volume of ethanol) at 50,000 particles/sec. Note: When creating aerosols, which could contain Glo-Germ particles, it is recommended the operator

wear a 0.2 μm particle mask.

- Place the AeroTech concentrator at various places outside of the HEPA filtered enclosure. Allow collection for at least 5 minutes.
- Turn off vacuum to AeroTech concentrator and remove slide. Put in a fresh slide and continue collecting aerosol with the smoke evacuation system and HEPA filtered enclosure turned off for 5 minutes (positive control). Stop sample acquisition and remove waste catcher plastic sleeve.
- Examine glass slides for bright green fluorescence using a fluorescent microscope. When present, Glo-Germ particles form a pattern as directed by the holes in the plate cover.
- Scan the entire slide on 10x and count all Glo-Germ particles. The positive control slide can be used as a reference to distinguish between fluorescent debris and actual Glo-Germ particles.
- Acceptable Tolerance: No Glo-Germ particles detected. The positive control slide must contain greater than 100 particles after 5 minutes of active air sampling.

If these tolerances are not met, infectious cell sorting is not permitted.

10. PROCEDURE AFTER SORTING FAILURE

- Stop the stream by deactivating the RUN button
- Wait 5 minutes for aerosols to settle down
- Open the splash shield and remove the collection tubes
- Remove the sample tube
- Perform a sample line back flush by pressing BACK FLUSH
- Decontaminate the collection chamber with 1% Virkon
- If necessary debubble the system
- Start the stream again by pressing the RUN button
- If the blockage persists, clean the nozzle
- Stop the stream by deactivating the RUN button
- Unscrew the nozzle
- Place the nozzle in a Falcon tube with 70% ethanol
- Sonicate the nozzle for 1 min
- Reinstall the nozzle
- Debubble the system
- Start the stream by pressing the RUN button

REFERENCES

1. International Society for Analytical Cytology Biosafety Standard for Sorting of Unfixed Cells. Cytometry Part A 71A:414-437. 2007

Table 1
Infectious Agents Associated with Laboratory-Acquired Infections due to Manipulation with Biological Samples

Agent	Source of infection	Species	Route of infection	Biosafety level: Practices, safety equipment, and facilities
Hepatitis B, C, D virus	Blood, cerebrospinal fluid, urine, tissues	Human, naturally or experimentally infected primates	Inoculation, exposure of mucosal membranes to aerosols, broken skin	BSL2, BSL3 in case of aerosol production, large quantities or high concentrations
Herpes virus simiae (B virus)	Primary cultures (Rh monkey kidney cells)	Macaque or human	Inoculation, aerosol inhalation minimal	BSL3, BSL4 for large quantities or high concentrations
Herpes simplex 1,2 varicella virus	Ubiquitous	Opportunistic pathogen in immunocompromised host	Direct contact or aerosol inhalation	BSL2
Cytomegalovirus; Epstein–Barr virus (EBV)	Blood, tissues, EBV-transformed cell lines	Human	Inoculation, exposure of mucosal membranes to aerosols, broken skin	BSL2
Herpes 6,7 virus	Blood, bronchoalveolar lavage	Human	Risk not known	BSL2
Influenza virus	Bronchoalveolar lavage, respiratory tissues	Human, naturally or experimentally infected animals	Aerosol inhalation	BSL2
Lymphocytic chorio-meningitis virus	Blood, cell cultures, nasopharynx secretions, bronchoalveolar lavage, tissues	Nude mice, SCID mice, naturally infected macaques or marmosets, possibly man	Inoculation, exposure of mucosal membranes to aerosols, broken skin, well documented transmission by aerosol inhalation	BSL2, BSL3 in case of aerosol production, large quantities or high concentrations
Poliovirus	Fluids, tissues, respiratory secretions	Naturally or experimentally infected nonhuman primates, transgenic mice	Ingestion, parenteral inoculation	BSL2 practices by vaccinated personnel, use WHO guidelines for establishing BSL2/polio and BSL3/polio laboratories after wild polio has disappeared, when oral vaccination has stopped BSL4 for work with wild polio
Pox viruses; genetically engineered recombinant vaccinia virus	Lesion fluid, tissues, respiratory secretions, bronchoalveolar lavage	Infected volunteers or animals	Inoculation, exposure of mucosal membranes to aerosols, broken skin	BSL2 practices by vaccinated personnel
Human immunodeficiency virus (HIV-1, 2) Retroviral vectors containing full-length infectious genomes	Blood, body fluids, tissues	Human	Inoculation, exposure of mucosal membranes to aerosols (containing concentrated virus), broken skin	BSL2, BSL3 in case of aerosol production, large quantities or high concentrations
Simian immunodeficiency virus (SIV)	Blood, body fluids, tissues	Macaque	Inoculation	BSL2, BSL3 in case of aerosol production, large quantities or high concentrations
Vesicular stomatitis virus	Blood, body fluids, tissues	Infected animals, humans	Exposure to infectious droplets or aerosols, direct skin and mucosal membrane exposure	BSL3, BSL2 for laboratory-adapted strains with demonstrated low virulence

Table 1
Infectious Agents Associated with Laboratory-Acquired Infections due to Manipulation with Biological Samples (continued)

Agent	Source of infection	Species	Route of infection	Biosafety level: Practices, safety equipment, and facilities
HTLV-1, 2 virus	Blood, body fluids, tissues	Human	Inoculation, exposure of mucosal membranes to aerosols (containing concentrated virus), broken skin	BSL2, BSL3 in case of aerosol production, large quantities or high concentrations
Coxiella burnetii	Blood, urine, tissues	Infected animals, humans	Inoculation, exposure to infectious aerosols	BSL2, BSL3 for cell culture and manipulation of tissues
Rickettsia prowazekii	Infected tissues	Naturally infected, nonhuman primates	Inoculation, aerosol inhalation	BSL2, BSL3 for tissue cultures of infected cells
Brucella	Blood, cerebrospinal fluid, tissues	Human, experimentally infected animals, sheep	Inoculation, direct skin contact	BSL2, BSL3 for tissue cultures of infected cells
Bacillus anthracis	Blood, cerebrospinal fluid, pleural fluid	Naturally and experimentally infected animals	Exposure of intact and broken skin, inoculation	BSL2, BSL3 in case of aerosol production, large quantities or high concentrations
Chlamydia psittaci	Blood, tissues	Birds, human	Exposure to infectious aerosols and droplets	BSL2, BSL3 in case of aerosol production, large quantities or high concentrations
Leptospira interrogans	Blood, tissues	Infected animals, human	Inoculation, skin and mucous membrane contact	BSL2
Listeria monocytogenes	Blood, cerebrospinal fluid	Naturally or experimentally infected animals	Ingestion, eye and skin exposure	BSL2
Mycobacterium atypicum	Bronchoalveolar lavage, lesion tissues	Human	Inoculation, direct skin contact, aerosol inhalation	BSL2
Mycobacterium tuberculosis	Gastric lavage, cerebrospinal fluid, pleural fluid, urine	Human, naturally infected primates	Aerosol inhalation	BSL3
Neisseria gonorrhoeae	Sinovial fluid, urine, cerebrospinal fluid	Human	Inoculation, direct skin contact	BSL2
Neisseria meningitidis	Pharyngeal exudates, bronchoalveolar lavage, cerebrospinal fluid, blood	Human	Inoculation, direct skin contact, aerosol inhalation	BSL2, BSL3 in case of aerosol production or high concentrations
Salmonella Salmonella typhi	Blood	Human	Inoculation, direct skin contact	BSL2, BSL3 for large quantities
Treponema pallidum	Lesion fluid	Humans with primary and secondary syphilis	Inoculation, direct skin contact, aerosol inhalation	BSL2
Toxoplasma, Trypanosoma, Leishmania, Plasmodium	Blood	Humans or experimentally infected animals	Inoculation, aerosol inhalation	BSL2
Blastomyces, Coccidioides, Histoplasma	Tissues	Infected animals	Inoculation, aerosol inhalation	BSL2, BSL3 for cultures containing Coccidioides, Histoplasma

This table was adapted from US HHS Publication: Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999.