Flow Cytometer and Cell Sorter Guidelines

Introduction
The National Institutes of Health (NIH) developed a policy, the NIH Biosafety Policy for Cell Sorters, for NIH facilities that utilize cell sorters. It is understood that this policy may become guidelines for NIH funded facilities in 2016. In anticipation of these guidelines becoming required for all NIH funded proposals, the Northeastern University Institutional Biosafety Committee has voted to accept these guidelines to begin the transition over the next two years.

Flow cytometers are a class of automated instruments that provide quantitative data on properties of individual cells. These instruments measure cell size and the amount of cell components such as DNA, messenger RNA, surface receptors and intracellular proteins. Flow cytometers make these measurements by taking a suspension of cells and streaming the individual cells past a laser beam and measuring the light scatter or fluorescence of the material in solution. There are two general types of flow cytometers:

1. Analytical – the material is sent past the laser in a closed system for analysis and the sample is disposed of. The generation of aerosols is less likely given the closed nature of the system.
2. Cell sorters (also known as jet-in-air or stream-in-air cell sorters) – material is separated into containers based on properties of each cell in order to prepare the material for culture or future work. The generation of aerosols is highly likely since the fluid containing the cells is ejected from a small opening at high pressure and deflected off a solid surface.

Purpose
The purpose of this document is to provide safety guidelines for the installation, maintenance and use of flow cytometers and cell sorters, specifically fluorescence activated cell sorters (FACS). Additionally this document should be used as a reference for the generation of standard operating procedures for the maintenance and use of flow cytometers and cell sorter at Northeastern University.

Responsibilities
Each Principal Investigator (PI) is responsible for completing a risk analysis and written Standard Operating Procedures (SOP) to implement safety precautions
Risks and practice. The Biosafety Program Manager must approve these before work with the flow cytometer or cell sorter may begin. Assistance with the development of the SOP can be requested from the Biosafety Program Manager in the Office of Environmental Health and Safety.

**Risk Assessment**

Prior to cell sorting, a written risk assessment must be conducted and risk controls must be established in order to determine the appropriate practices and procedures. When completing the risk analysis, follow the five steps as outlined in the NIH Biosafety Policy for Cell Sorters:

1. Identification and evaluation of the agent’s hazards.
2. Identification of laboratory procedures and the hazards associated with those procedures.
3. Determination of the biosafety level.
4. Complete an evaluation of staff proficiency with the equipment and determine the integrity of the associated safety equipment.
5. Approval of the risk assessment and procedures by the Biosafety Program Manager.

The risk group of the agent can be determined using a variety of resources including the Biosafety in Microbiological and Biomedical Laboratories (BMBL). As indicated in the NIH Biosafety Policy for Cell Sorters, materials that are considered a low risk to humans and designated as BL2 agents under normal practices and procedures may be classified as BL2 with enhanced precautions or BL2 + (BL2 containment with BL3 work practices and procedures) because of the potential splash hazard, droplet and aerosol formation.

**Hazards**

Cell sorting is considered a procedure hazard in the laboratory because there is a higher potential for exposure to splashes and aerosols during the use of this equipment. Exposure to potentially infectious and hazardous materials may occur by:

- Handling the material prior to use in flow cytometer or cell sorter
- Aerosols and droplet formation in the flow cytometer or cell sorter

Animal specimens may contain animal pathogens, infectious or zoonotic agents or unknown pathogens. Transmission of these pathogens can occur through:

- Inhalation
- Percutaneous injury
- Mucous membrane exposure to aerosols or droplets.

Unfixed human materials including human cells, tissues, human materials from patients, etc. can harbor bloodborne pathogens including, but not limited to:
• Hepatitis B
• Hepatitis C
• HIV

Unfixed infectious agents (i.e. cultures of bacteria, etc.), animal tissues and recombinant nucleic acid material (rDNA, rRNA, viral vectors, transgenes, etc.) could result in the transmission of known or unknown pathogens through exposure.

Exposure to dyes used in staining that may be carcinogenic, mutagenic or toxic is also a concern. Appropriate procedures must be developed to determine the potential hazards of the specimens and to plan for waste management, aerosol containment and equipment maintenance.

Many of these instruments utilize lasers and most have a safety device that prevent user exposure to the beams and are difficult to disable during normal use. Cell sorters present a laser hazard if the interlocks are defeated or if the safety covers are removed.

**Standard Operating Procedure (SOP) and Registration Requirements**

**Training Requirements**

In accordance with the OSHA Bloodborne Pathogens Standard, Northeastern University’s Biosafety and Exposure Control Plans, personnel working with human materials and potentially infectious agents must complete appropriate training at the interval required by the regulation and university practices. Initial training must be completed in the classroom and annual refresher training may be taken online.

**SOP Development and Approval**

Principal Investigators using a cell sorter with unfixed material must generate a complete SOP for utilizing the equipment. This SOP must include:

- Requirements of the laboratory space
  - Negative air flow
  - Equipment in separate, lockable room where no other work is performed (BL 2+)
- Procedures for preparing for the sort
  - Empty waste and check fluids
  - Containment testing
  - Preparation of reagents for decontamination
  - Warning signage on door
- Safety equipment and practices
  - Filter change frequency
  - Aerosol containment
  - Personal protective equipment
• What to do in the event of a nozzle obstruction
  o If the nozzle opening is partially obstructed, the stream diverges and there is a higher risk for aerosols to form in the instrument
• Disinfection and the decontamination procedures

Standard requirements should adhere to those set forth in the NIH Biosafety Policy for Cell Sorters and the SOPs included in this document may be used as a template. For assistance in the development of the SOP, contact the Biosafety Program Manager. The Biosafety Program Manager must approve of the cell sorter or flow cytometer protocol and materials before work can commence.

**Personal Protective Equipment (PPE)**
Cell sorter and flow cytometer operators must wear the appropriate PPE for the material being analyzed and the procedures used. At a minimum, operators must don gloves, a lab coat and eye protection. Depending on the material being analyzed and the equipment design, further PPE, such as respiratory protection, may be utilized.

For more information about respiratory protection, please contact the Office of Environmental Health and Safety at 617.373.2769 or ehs@neu.edu.

**Aerosol Containment, Equipment Validation and Preventative Maintenance**
According to the NIH Biosafety Policy for Cell Sorters, an aerosol management system should be used at all biosafety levels. This system typically consists of an evacuation pump connected to a HEPA or ULPA filter. When working with BL2 or BL2 + materials, this containment system must be validated monthly and when the filters are changed. This validation can be accomplished by using “GloGerm” particles or another approved technique. Routine preventative maintenance must be done in accordance with the operator's manual and all equipment must be certified annually. Depending on the material, the equipment may need to be in a biosafety cabinet or similar containment (e.g. BioBubble).

**Documentation Requirements**
All work using flow cytometers and cell sorters must be documented in a logbook and records must be maintained. This log should include:
• Validation of the aerosol management system results
• Equipment usage
  o Date
  o User
  o Type of material
  o Length of usage (for aerosol management system)
  o Nozzle failure or clogs
  o Disinfectant used and contact time
• Records of routine preventative maintenance
• Record of annual certification

Resources

Biosafety in Microbiological and Biomedical Laboratories 5\textsuperscript{th} Edition
http://www.cdc.gov/biosafety/publications/bmbl5/

NIH Biosafety Policy for Cell Sorters