POLICY FOR FLUORESCENCE-ACTIVATED CELL SORTING (FACS)

Procedure: 2.18 **Effective: 09/06/2016**
Version: 1.1

A. Purpose

This policy serves to provide guidance for the safe installation and use of fluorescence-activated cell sorters (FACS). FACS analysis poses a unique hazard in the laboratory due to the relatively high potential for the aerosolization of materials that are subject to processing. Many unfixed cells may pose a potential hazard in the form of known and unknown pathogens, viral vectors used to transduce cells, genomic sequences of infectious agents, and chemical mutagens. When potentially infectious materials are processed in FACS analysis, special procedures and containment are often required to mitigate this unique hazard.

B. Applicability/scope

Columbia University serves as a home for several FACS "core facilities" that process samples for the broader University community. These are either departmental resources (e.g. Microbiology and Immunology) or associated with Howard Hughes Medical Institute or tenant (NY Stem Cell Foundation) Investigators, and as such, may be available as a service for use by other laboratories. Detailed standard operating procedures (SOP) are a critical necessity for FACS analysis activities that could pose a potentially significant risk to human health and the environment. This policy serves as basic guidance for safety measures in these core facilities, and may be used to aid in the creation of lab-specific SOPs, along with the guidance documents provided below (**Section K: References**). This document, along with the additional resources cited and direct consultation with Environmental Health and Safety (EH&S), should be utilized for the design of any laboratories that are to house FACS equipment.

C. Responsibilities

1. FACS Facility Managers

Maintenance of, and adherence to, lab-specific SOPs are the responsibility of the core facility managers, as these procedures will differ between facilities depending on the equipment used and the nature of the materials processed. Facility managers are required to triage all samples provided by laboratories for processing, and to perform risk assessments to determine if the materials can be processed safely. Screening can be accomplished via the use of a submission form accompanying samples for sorting or other similar means as determined by the facility's SOP. Facility managers are to consult with EH&S when assistance is needed with any risk assessment, or if a risk assessment determines that it may not be possible to safely process a sample. Facility managers are to promptly report any accidents or exposures to EH&S and seek medical attention (see **Section F: Emergency contacts**).

2. **Department Administration**
   Departments that house FACS instruments and operations are responsible for providing facilities that meet design and engineering standards set forth in the ISAC and NIH policies. In addition to the engineering controls, appropriate personal protective equipment (PPE) as mandated by the biosafety level required for the material that is processed must be made available.

3. **Environmental Health and Safety (EH&S)**
   EH&S:
   - **a.** provides technical assistance with risk assessments as well as consultation regarding appropriate safety precautions for FACS processing of potentially infectious samples.
   - **b.** provides guidance regarding the design of laboratories housing cell sorters.
   - **c.** is the custodian of this policy. The policy is to ensure that FACS processing requirements at Columbia University reflect accepted safety standards.
   - **d.** is available for emergency response in the event of any release of infectious material.

4. **FACS Facility Users and Principal Investigators (PIs)**
   Researchers at Columbia that submit materials to any of the core FACS facilities for processing are responsible for accurately and comprehensively completing the appropriate requisition forms for each facility and, prior to submission, for preparing samples in accordance with each facility's SOP.

   Any researcher that directly operates any equipment or devices in the core facilities must strictly adhere to any and all guidelines, rules, and standard operating procedures enforced by the respective core facility manager.

   Any Principal Investigators that operate their own FACS equipment are to consult with EH&S if assistance is needed with any risk assessment, or if a risk assessment determines that it may not be possible to process a sample safely. PIs are to develop their own SOPs in accordance with this policy and ensure that these procedures are enforced. PIs will not be subject to the requirements outlined in this policy for sample screening through written requisitions if all samples to be processed originate from their own laboratories, no external samples are processed, and their own SOP describes the nature of material that is sorted in their lab.

5. **Institutional Biosafety Committee (IBC)**
   The IBC is broadly charged with reviewing and approving work with recombinant DNA that occurs at Columbia University, and also provides high level review for other significant biosafety concerns, including FACS.

D. Definitions

AMS – Aerosol Management System

BMBL – *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition*
BSC – Biosafety cabinet
EH&S – Environmental Health & Safety
FACS – Fluorescence Activated Cell Sorting
IBC – Institutional Biosafety Committee
ISAC – International Society for Analytical Cytology
NIH – National Institutes of Health
PAPR – Powered Air-Purifying Respirator
PI – Principal Investigator
PPE – Personal Protective Equipment

E. Procedures

1. Risk Assessment

A thorough risk assessment must precede FACS processing of potentially infectious agents. This risk assessment should consist of: identification of the agent to be manipulated or source of the sample cells; description of the procedures to be performed (including sample preparation and manipulation); determination of the appropriate biosafety level for the procedures; evaluation of the competency of the personnel; and assessment of the suitability of the equipment and facilities to carry out the operations safely. Consult EH&S to review the determinations of risk assessments and to assist with any part of this process.

The selection of the appropriate biosafety level should be made during the course of the risk assessment. This decision may be made in consultation with the biosafety officer, and must be in keeping with the guidelines set forth in the references cited in Section K of this document, in particular BMBL 5th Ed. and the NIH Biosafety Policy for Cell Sorters. There can be unnatural routes of exposure in the laboratory setting that are generally not seen in nature. Because of the elevated risk of aerosol generation, the material from a given risk group used in FACS processing normally requires: operating procedures; PPE; and engineering controls above what would normally be required (see **Section I: Appendices, Table 1**). For example, human cell lines infected with influenza virus, which would normally be manipulated at BSL-2, would, when subject to FACS processing, be handled at BSL-2 with enhanced precautions (BSL-2+). This includes additional precautions beyond what is normally used to handle human cell lines, such as the use of respiratory personal protective equipment and a wrap-around gown or rear-closure lab coat.

Since some FACS facilities may not have the necessary infrastructure in place to handle potentially hazardous samples with the required elevated precaution, it is critical that risk assessments be properly performed to identify which materials can and cannot be processed safely. Each FACS facility will need to determine what biosafety level is appropriate, and in most cases will rely upon standard requisition forms (see **Section J: Forms**) for determining if a given sample can be safely processed.

2. Standard Operating Procedures (SOP)

The use of written standard operating procedures is central to the safe conduct of cell sorting. Because each FACS facility is unique in terms of its infrastructure, personnel, equipment, and materials processed, it is critical for each FACS facility manager to develop a customized written SOP. This SOP should account for the outcome of any risk assessment, the unique properties of each facility and equipment, and should ultimately serve to minimize or eliminate the risk of exposure to hazards. Include also procedures for restricting access to shared spaces during sorts, hazard communication signage and criteria for outsourcing potentially high risk sorts should also be included.

The *NIH Biosafety Policy for Cell Sorters* is an excellent resource and should be used as a starting point for development of any core facility-specific SOP. EH&S is available to assist with the development of these SOPs and should be consulted to review any facility- or equipment-specific SOP before it is finalized and put into practice.

3. Personal Protective Equipment (PPE)

PPE worn during cell sorting should be based on a thorough risk assessment and should adhere strictly to the established SOP for the facility. In general, PPE used for FACS facilities may need to be a level above the standard BSL-2 lab ensemble consisting of a cotton lab coat, safety glasses, and single-layer gloves. Facilities that need to operate at BSL-2 with enhanced precautions (BSL-2+) can meet this additional level of containment by keeping dedicated PPE within the space reserved for cell sorting. Additional PPE enhancements may include double layers of gloves, impermeable lab coats with a rear-closure or wrap-around designs, face shields/fully enclosed goggles, and/or respiratory PPE such as N-95 particulate respirators or powered air-purifying respirators (PAPR). See **Section I: Appendices, Table 1** for additional guidance on selecting PPE, or consult with EH&S.

4. Engineering Controls

FACS instruments perform analysis of cells, and are designed so that desired cells are isolated into droplets that are deflected electrostatically into open collection vessels. These cell sorters are known as “stream-in-air” or “jet-in-air” cell sorters, which describes the ejection of fluid from the nozzle into the open air. There is a high likelihood of aerosol production by cell sorters due to the mechanical action of a fluid exiting a small orifice at high pressure and then impacting a hard surface. Aerosol production is highest in the event of a partial obstruction of the nozzle orifice and the subsequent stream deviation. Therefore, use of this instrumentation with infectious or potentially infectious samples defines this process as a hazardous procedure. Safe FACS operations rely heavily upon several layers of engineering controls to protect personnel and the environment from exposure or release. These controls consist of a combination of an integrated aerosol management system (AMS), a biosafety cabinet (BSC) to contain the equipment, and a negative-pressure room with directional airflow away from non-containment areas to contain the device. As with any biocontainment facility, a hand-

washing sink is essential, and consideration should be given to the installation of "hands free" activation capability during the design of new facilities.

a. Aerosol Management Systems (AMS)

Many modern cell sorters are equipped with integrated AMS to contain and evacuate aerosols that may be generated during the course of sorting operations, or in the event of a nozzle clog that could lead to the generation of a large plume of aerosols. This system usually consists of an evacuator that creates negative pressure within the sort chamber and/or sort collection area of the cell sorter, which transports aerosols to a HEPA filter before exhausting to the room. While AMS provide an excellent built-in means of stopping aerosol releases at the source, it must be regularly validated to ensure proper operation. Any SOP should include steps for both evaluating and validating the operation of the AMS, if present. The *NIH Biosafety Policy for Cell Sorters* is an excellent resource and example SOP for AMS validation, though each FACS facility manager or any PIs operating their own FACS equipment will need to customize this procedure. The AMS must be correctly used in the context of the cell sorting procedure to ensure intended performance. This needs to be written into the SOP. Note, the absence of an AMS effectively limits a facility to BSL-1 operations.

b. Biosafety Cabinet (BSC)

Enclosing a cell sorter within a special Class II (NSF-49 certified) BSC is the most reliable way to reduce the potential for exposure in the event of a failure of the aerosol management system. Most cell sorter manufacturers also make BSCs that are specifically designed for the model of cell sorter they sell. For FACS facilities operating at BSL-2 with enhanced precautions (BSL-2+), the use of a BSC may eliminate the need to house the cell sorter in a dedicated room (away from any unrelated activities or personnel).

A qualified technician will need to install the cell sorter as well as the BSC, and then evaluate the function of the unit as a whole. Any BSC used to house a cell sorter must be 1) located away from strong air currents or high-traffic areas and 2) certified annually by a qualified technician.

c. Directional air flow

BSL-2 or BSL-2+ sorting operations should take place within a room that is subject to negative pressure with respect to nearby non-containment areas. The sorter should ideally be located in a dedicated space away from unrelated activities, equipment, and personnel. A visual monitoring device, such as a magnehelic gauge or a "ball-in-the-wall," may be employed to provide a visual indication of the direction of air flow.

5. FACS Requisition Screening

The proper management of any hazard relies heavily upon a risk assessment, and the first step of any risk assessment is the identification of the agent. In order to ensure that FACS facility personnel are able to work safely, FACS facility users must communicate the risk potential of their samples to the FACS facility manager when using a core facility. Investigators must clearly identify the contents of any sample submitted to the facilities. The communication shall take place through the use of the standard requisition form (**Section J: Forms**). EH&S may be consulted if there are any questions about completing this form.

F. Emergency contacts

Accidents or exposures must be immediately reported to EH&S at (212) 305-6780. After hours emergencies may be reported to Public Safety at (212) 305-7979. Potential exposures to infectious agents should receive medical evaluation and must be reported to EH&S using an accident report form: http://www.ehs.columbia.edu/Accident%20Report%20form.pdf. For details on health care providers see: http://www.ehs.columbia.edu/WhereToGoForMedicalAttention1.pdf

G. Medical Surveillance

1. Personnel with anticipated potential exposure to infectious materials should receive baseline medical surveillance (http://www.ehs.columbia.edu/WhereToGoForMedicalAttention1.pdf).
2. Personnel potentially exposed to infectious material should seek post exposure monitoring as per the Bloodborne Pathogens Exposure Control Plan (http://ehs.columbia.edu/BloodbornePathogensExposureControlPlan.html#exp).
3. Personnel that require the use of a respirator must be medically cleared and fit tested for the use of the respirator, and enrolled in the respiratory protection program (http://www.ehs.columbia.edu/RespiratoryProtectionProgram.html).

H. Recordkeeping

FACS facility managers are to maintain records of requisitions for at least two years.

I. Appendices

Table 1: Biosafety Level Determination for Cell Sorting*

	BSL-2	BSL-2 with enhanced precautions (during sorting operations)	BSL-3
Risk Assessment Condition	Uninfected non-primate	Non-infectious Human/NHP cells Infectious but with low risk assessment	Infectious samples with high risk assessment All samples containing known aerosol pathogens
Example sample type or agents[1]	Normal murine cells 3rd gen lentivirus (non-human cells)	Normal human blood Human cell lines[1] An example agent is: Influenza A[1] 2nd gen lentivirus or 3rd gen lentivirus in human cells	Example agents include[1]: Mycobacterium tuberculosis; Monkeypox
Containment system validated	Periodically (monthly or with filter change)	Periodically (monthly or with filter change)	Before every sort
Aerosol containment operational	Required	Required	Required
N-95, N-99, or N-100 respirator	Optional	Required[2]	N/A
PAPR	Optional	Optional	Required
Eye protection	Safety glasses	Face shield or safety goggles	N/A
Lab coat	Front closure lab coat	Wrap-around rear closure	Coveralls
Separate room and environmental controls	Optional	Required or limited access to room[3]	Required[4]

***The above table and related footnotes are excerpted from the NIH Biosafety Policy for Cell Sorters (NIH, 2012).**

[1]Example Sample type or Agents – the samples and/or agents listed represent only a partial list of agents which may be included in each category. A risk assessment should be conducted for all samples/agents prior to sorting, and the appropriate biosafety level determined in collaboration with safety specialists, subject matter experts and the NIH IBC. For additional information please consult the following web sites: http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php; http://www.cdc.gov/od/OHS/biosfty/bmbl5/bmbl5toc.htm

[2]Respirators must remain on during all procedures associated with sample manipulation, including sample tube cap removal and loading of sample on instrument, or when removing collection tubes or other procedures where the sort or collection chamber is opened. Note that respirator protection may otherwise be removed during the sorting process providing the aerosol management system is active and all sort chamber and collection chamber doors are closed. For human pathogens that are classified as BSL2 and are not respiratory hazards, but which may pose a risk if exposed to mucous membranes, only mucous membrane protection is required. Examples of agents in this category include Leishmania and toxoplasmosis in murine cells

[3]Enclosure of the cell sorter within a Class II BSC (NSF-49 certified) may abrogate the need to house the sorter in a separate room within the BSL2 lab space; PPE (as detailed above) is optional, but strongly encouraged for the operator during procedures requiring manipulation of instrument. Cell sorters located within a shared laboratory may be operated under BSL2 with enhanced precautions if during the operation of the sorter, access to the room is limited and PPE as detailed above is worn by all occupants

[4]Enclosure of cell sorter within a Class II (NSF-49 certified) BSC required

J. Forms

FACS of Potentially Biohazardous Materials Request Form

Date: _____ Project Title_____

PI UNI:_____ Office phone:_____ Cell phone (in case of emergency): _____

Personnel (anyone involved with working with or preparing the cells for FACS)

	Name	UNI	Lab phone	Cell phone (in case of emergency)
1.				
2.				
3.				
4.				

Description of Project (species, cell type, cell line/primary specimen):

_____

_____

_____

_____

Was this protocol reviewed by the IRB, IACUC or IBC? Include numbers of any Animal Protocols or Hazardous Materials Appendices A, B or C.

_____

What species are the samples to be sorted derived from?

_____

Are the samples to be sorted known or suspected to be infected with any of the following? (indicate Yes or NO): HIV _____ HCV _____ HBV _____

Are the samples to be sorted known to contain any infectious agents?

_____

Were the cells transformed using a virus such as EBV, HTLV-1, etc?

_____

Were the cells engineered with adenovirus, retrovirus, lentivirus? List the virus and give a brief description of the system used.

_____

_____

Will the sample be fixed prior to submission to the flow cytometry core facility? Yes No

If yes, describe the fixation protocol in detail (*e.g., fixative, fixative concentration and exposure time*).

_____

I have read the above questions carefully and certify the information provided to be correct. If my experimental setup has changed in any way from the information that has been provided, I will submit an updated FACS of Potentially Biohazardous Materials Request Form.

PI name: _____PI signature: _____

This section is for use by flow cytometry core facility

Date reviewed: _____ Person reviewed: _____

Issues or concerns raised by review:

_____

_____

Accept or reject the request:____ Biosafety level assigned: _____ Project number: _____

Date of email sent to PI: _____ Date of email sent to EH&S: _____

Signature of flow cytometry core facility staff: _____

K. References

Biosafety in Microbiological and Biomedical Laboratories, 5th Ed. (2009, December). U.S. Department of Health and Human Services.

NIH Biosafety Policy for Cell Sorters. (2012). National Institutes of Health.

Holmes, K. et al. (2014, May) International Society for the Advancement of Cytometry Cell Sorter Biosafety Standards. Cytometry A. 85(5): 434–453.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4117398/pdf/nihms592145.pdf

Schmid, I. et al. (2007, January). International Society for Analytical Cytology (ISAC) Biosafety Standard for Sorting of Unfixed Cells. *Cytometry Part A*, 71a:414-437.

L. Acknowledgements

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