NIH Policy Manual

3038 - Working Safely with Cell Sorters

Issuing Office: OD/OM/ORS/SR/DOHS **Phone:** (301) 496-2960

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Transmittal Notice

1. **Explanation of material transmitted:**

   This chapter establishes the NIH policy for the installation and operation of the class of laboratory instruments known as cell sorters or fluorescent activated cell sorters. It provides guidance and minimum standards to protect the safety of employees/workers, the public, and the environment while working with cell sorters.

1. **Filing Instructions:**

   Insert: NIH Manual Chapter 3038, dated 09/26/2017

PLEASE NOTE: For information on:

- Content of this chapter, contact the issuing office listed above.
- NIH Policy Manual, contact the Division of Management Support, OMA at (301) 496-4606 or enter this URL: https://oma.od.nih.gov/DMS/Pages/Manual-Chapters.aspx

A. Purpose

By design, stream-in–air cell sorters produce aerosols. Therefore, the use of these instruments with certain biological agents constitutes a potential procedure hazard. This chapter establishes requirements for the design of laboratories housing cell sorters, the creation of laboratory or instrument-specific Standard Operating Procedures (SOPs), and the procedures for the safe operation of cell sorters and validation of their aerosol containment systems.

B. Scope

The objectives and responsibilities set forth in this manual chapter are applicable to all NIH employees in all Institutes and Centers. NIH employees will comply with this policy and perform their duties in the safest possible manner.

C. Background

Flow cytometric cell sorting has become an important technology in basic and clinical research laboratories. However, samples that are sorted may contain infectious biological agents, and standard procedures must be implemented to minimize risk of exposure to these potentially hazardous agents. In addition, these standard procedures must be consistent with NIH Manual Chapter 3035.

Laboratory procedures that generate aerosols are classified as the most important operational risk factor supporting the need for containment equipment and facility safeguards. The likelihood of aerosol production by cell sorters is high due to the possibility of fluid exiting a small orifice (usually 70µm) µm at high pressure (up to 70psi) impacting a hard surface. Aerosol production is highest in the event of a partial obstruction of the nozzle orifice and subsequent stream deviation. In recognition of this risk of aerosol exposure when sorting potentially infectious material, the NIH Biosafety Policy for Cell Sorters was approved in a memo to the Director, Division of Occupational Health and Safety (DOHS), ORS, in 2012.

The fundamental objectives of any laboratory biosafety program should be containment of hazardous materials, and the development and implementation of procedures designed to reduce exposure based upon a thorough risk assessment. This policy is meant to reduce or eliminate exposure of the outside environment and laboratory personnel to potentially hazardous agents during the operation of cell sorters.

This policy is derived from established biosafety principles as outlined in the Biosafety in Microbiological and Biomedical Laboratories (BMBL), current edition , and the current International Society for the Advancement of Cytometry (ISAC) Cell Sorter Biosafety Standards.

This policy supplements other safety policies at the National Institutes of Health (NIH), and does not reduce or alter the requirements of any other policies.

D. Policy

All laboratories in the NIH Intramural Research Program utilizing Cell Sorters follow procedures defined in this NIH Manual Chapter. These procedures (outlined in Section H) consist of six sections, 1) Risk Assessment; 2) SOP Development; 3) Room Design; 4) Cell Sorter-Specific Safety Equipment and Practices; 5) User-Specific Safety Equipment; and 6) Disinfection.

E. References

References and copies of registration forms are available from the DOHS website. Occupational Safety and Health Administration (OSHA) references are available on the OSHA website. Additional references include:

1. Holmes KL. Characterization of aerosols produced by cell sorters and evaluation of containment. Cytometry A 2011;79:1000-1008.
2. NIH Policy Manual, Chapter 3035: Working Safely with Potentially Hazardous Biological Materials
3. Holmes KL, Fontes B, Hogarth P, Konz R, Monard S, Pletcher CH Jr, Wadley RB, Schmid I, Perfetto SP. International Society for the Advancement of Cytometry cell sorter biosafety standards. Cytometry A 2014;85:434-453.
4. Oberyszyn AS. Method for visualizing aerosol contamination in flow sorters. Curr Protoc Cytom 2002;Chapter 3:Unit 3.5.
5. Perfetto, S. P., et al. "Novel Impactor and Microsphere-Based Assay Used to Measure Containment of Aerosols Generated in a Flow Cytometer Cell Sorter." Cytometry A. 2018 Dec 18. doi: 10.1002/cyto.a.23680.
6. Rutala WA, Weber DJ, HICPAC. Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008. 2011.
7. Biosafety in Microbiological and Biomedical Laboratories - Centers for Disease Control and Prevention/National Institutes of Health. The current edition is available at the DOHS Publications page.
8. NIH Bloodborne Pathogen Exposure Control Plan for Non-Hospital Personnel. Prepared in compliance with 29 CFR 1910.1030.
9. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). Federal Register, July 5, 1994 (59 FR 34496). Amendment - Federal Register, FR, January 5, 2001 (66 FR 1146) and all current amendments.
10. Occupational Exposure to Bloodborne Pathogens. Occupational Safety and Health Administration Standard 29 CFR 1910.1030, 66 FR 5325 January 18. 2001.
11. NIH Policy Manual, Chapter 1743: Keeping and Destroying Records

F. Definitions

1. **Flow Cytometric Analyzer** - Scientific instrument used to characterize cells or particles in a fluid stream, based upon their measured fluorescence and light scatter characteristics, but are incapable of sorting the cells.
2. **Fluorescence** - activated Sorter (FACS) - FACS is a trademarked name for BD Biosciences instruments.

   Note: The scope of this document is for Cell Sorters**, not analyzers**. The SOP's used as examples in this document are for BD FACS Aria cell sorters, hence the use of the name FACS. **It is important to distinguish a Cell Sorter from a Flow Cytometric Analyzer, since the risk of aerosol production from analyzers is much lower.**

1. **Cell Sorter** - Scientific instrument used to isolate cells or particles based upon their measured fluorescence and light scatter characteristics. There are two classes of Cell Sorters: electrostatic droplet-based (also known as jet-in-air or stream-in-air) and mechanical cell sorters. Electrostatic droplet-based sorters employ a liquid stream at high (up to 70 psi) pressure, carrying cells through a nozzle. The stream is not confined

and therefore is open to the air. Mechanical sorters utilize fluid streams that are confined within tubing or microfluidic channels. The term cell sorters used in this Chapter refer to electrostatic droplet-based sorters.

2. **Biosafety Levels** - A biosafety level is the level of the biocontainment precautions required to work with dangerous biological agents in an enclosed facility. The levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4). In the United States, the Centers for Disease Control and Prevention (CDC) have specified the requirements for each of these levels.
3. **Infectious Biological Agent** - A microorganism (including, but not limited to, bacteria (including rickettsiae), viruses, fungi, or protozoa) or prion, whether naturally occurring, bioengineered, artificial, or a component of such microorganism or prion that is capable of causing communicable disease in a human, animal, or plant.
4. **Personal Protective Equipment (PPE)** - Items of clothing (i.e. lab coats, shoe covers, face masks, gloves, etc.) or equipment (i.e. face shields, eye goggles, etc.) designed to prevent or limit exposure to potentially harmful agents.
5. **Standard Operating Procedure (SOP)** -Written procedures that describe, in detail, how to perform a particular task or overall duty/responsibility.
6. **Mucous membrane protection** - A device or combination of devices, such as a full face shield, surgical face mask combined with form fitting goggles or approved protective glasses, etc., which protect the mouth, nose and eyes from splash or droplet contamination.
7. **NIH Facility -** A facility owned, operated or leased by the NIH.
8. **Cell Sorter in certified Biological Safety Cabinets (BSC) -** Class II BSC: manufactured to meet functional certification criteria for personnel and product protection as defined by NSF 49; Class I BSC: manufactured to meet functional certification criteria for personnel protection as defined by the BMBL and have an inward airflow velocity of 100 linear feet per minute. High Efficiency Particulate Air (HEPA) filters are to be tested for leakage annually.
9. **Select Agents** – Select Agents are bio-agents which have been declared by the U.S. Department of Health and Human Services (HHS) or by the U.S. Department of Agriculture (USDA) to have the "potential to pose a severe threat to public health and safety". These bio-agents are divided into three broad categories: 1) HHS select agents and toxins (affecting humans); 2) USDA select agents and toxins (affecting agriculture); and 3) Overlap select agents and toxins (affecting both).
10. **NIH Institutional Biosafety Committee (IBC)** - The NIH Institutional Biosafety Committee reports to the Director, NIH or his or her designee on matters pertaining to the control of biological hazards. The NIH IBC is the primary reviewing and biosafety approval body for all proposed research associated with the intramural use of microbiological agents and recombinant or synthetic research subject to the NIH Guidelines. The IBC also serves as an advisory body to the Division of Occupational Health and Safety (DOHS), ORS.
11. **Human Pathogens** - Human Pathogens are agents (such as viruses, bacteria, prions, or fungi) that cause disease in humans.
12. **High Efficiency Particulate Air (HEPA) filter** -A throwaway, extended-media, dry type filter with a rigid casing enclosing the full depth of the pleats. The filter shall

exhibit a minimum efficiency of 99.97% when tested at an aerosol of 0.3 µm diameter.

13. **Agent Risk Group**-The classification of infectious 2microorganisms according to its capability to infect and cause disease in a susceptible human or animal host, its virulence as measured by the severity of disease, and the availability of preventative measures and effective treatment for the disease. Four Risk Groups are defined in the BMBL, ranging from least likely to cause human disease (Risk Group 1) to highly likely to cause serious or lethal disease (Risk Group 4).

G. Responsibilities

1. **Director, NIH:** Through DOHS and the Deputy Director for Intramural Research (DDIR), provides executive leadership in the development and implementation of biological safety policies, standards and procedures applicable to the NIH.
2. **Deputy Director for Intramural Research (DDIR):** The DDIR is the principal liaison with the NIH intramural research community regarding safety and health matters. The DDIR receives safety policies approved by ORS and communicates them to the IC Scientific Directors. Further, the DDIR raises safety concerns to ORS as they are brought to the DDIR's attention from the intramural research community.
3. **Designated Agency Safety and Health Official (DASHO):** The Institutional Official responsible for management and administration of the NIH occupational safety and health program. This authority is delegated by the Director, NIH.
4. **The Division of Occupational Health and Safety (DOHS):** The DOHS provides the necessary staff to effectively administer a comprehensive occupational safety and health program and it is responsible for:

    a. Providing general guidance on the selection and use of personal protective equipment (PPE) for laboratory personnel utilizing cell sorters. Specific PPE guidance shall also be provided for work involving infectious diseases, hazardous chemicals, etc., that may require additional PPE. If respirators are occupationally required, DOHS will select and approve them. Supervisors are not authorized to select or recommend the use of respiratory protection.
    b. Providing guidance in performing risk assessment to PI's and employees operating cell sorters, including the determination of appropriate biosafety level appropriate for the type of samples and/or agents that will be sorted. Contact the Safety and Health Specialist from the DOHS home page.

5. **Institute and Center (IC) Scientific Directors:** Scientific Directors are responsible for ensuring full compliance with this policy within the IC.
6. **Principal Investigator (PI) or Immediate Supervisor (IS)** is responsible for:

    a. Ensuring compliance from all personnel that work with Cell Sorters with all provisions of this Manual Chapter and any other special requirements or procedures specific to the facility within which they are working.
    b. The development and annual review of the Standard Operating Procedures (SOPs) for the Cell Sorting laboratory in compliance with this Manual Chapter. **The SOPs should be reevaluated at least on an annual basis or whenever**

there is a change in instrument configuration that may affect biosafety.

7. **Employees:**

    a. Employees must comply with the provisions of this Manual Chapter and the established Standard Operating Procedures of the Cell Sorting Laboratory. Employees are responsible for using prescribed personal protective equipment during performance of work.

H. Procedures

I. Risk Assessment

A risk assessment should be conducted for all samples/agents prior to sorting, and the appropriate biosafety level determined in collaboration with DOHS Safety and Health Specialists, subject matter experts, and the NIH Institutional Biosafety Committee (IBC). For additional information, consult DOHS Publications page. The purpose of a risk assessment is to recognize and identify hazards and measure the risk or probability that something will happen because of that hazard. The results of a comprehensive risk assessment determine the appropriate procedures and practices for cell sorting. The designation of safety measures is dependent upon the risk and the severity of the consequences if exposure occurs. Risk analysis takes into account the Risk Group of the agent and the procedures performed with the agent.

Risk Assessment consists of five steps:

1. Identify and evaluate agent hazards
2. Identify laboratory procedure hazards
3. Make final determination of biosafety level (BSL) (See Appendix I)
4. Evaluate proficiencies of staff and integrity of safety equipment
5. Review risk assessment with DOHS Safety and Health Specialist, or NIH Institutional Biosafety Committee.

The Risk Group of a given agent can be determined from a variety of sources, most notably the current edition of the Biosafety in Microbiological and Biomedical Laboratories, (BMBL). Cell sorting is considered a laboratory procedure hazard because of the potential for aerosol and/or slash exposure. Agents that may be worked with at BSL-2 under normal laboratory procedures and practices, may require greater precautions as defined in this document as BSL-2 with enhanced precautions. Due to the risk of aerosol exposure in cell sorting, an aerosol management system is required at all biosafety levels and usually consists of a sort chamber evacuation pump equipped with a High Efficiency Particulate Air (HEPA) filter. All aerosol management systems require validation (as indicated below), although the frequency of testing increases with increased biosafety levels.

II. Standard Operating Procedure (SOP) Development for Cell Sorter Laboratories

An important outcome of any risk assessment process is the creation of standard operating procedures (SOPs). An SOP must take into account hazards (agents and laboratory procedures) and specify practices and procedures designed to minimize or eliminate exposures to those hazards. For cell sorters, the design of the instrument, especially containment or aerosol evacuation components, must be considered in the development of the SOP. Each instrument must be evaluated for deficiencies in containment or aerosol evacuation design and appropriate procedures adopted to minimize risk. An important example of this is that most cell sorters do not possess an interlock designed to prevent the operator from opening the sort chamber after a nozzle obstruction with subsequent stream deviation. Therefore, the SOP should clearly address the procedures for evacuating the sort chamber of aerosols prior to opening the sort chamber, including a stated time period to wait after a clog induced stream deviation.

The general considerations for SOP development are outlined below:

1. Preparation before the sort

    a. Check fluids, empty waste
    b. Cover control surfaces with plastic wrap, including keyboards and mouse (or use washable keyboards).
    c. Perform containment testing
    d. Verify any automated decontamination functions
    e. Preparation of disinfectant solutions
    f. Sample preparation, i.e. staining, centrifugation, pipetting or manipulations that may generate aerosols should be performed in a manner to maximize containment and protect the worker

2. Procedures in the event of a nozzle obstruction

    a. Turn off stream
    b. Evacuate sort chamber prior to opening; increase Aerosol Management System (AMS) evacuation rate
    c. Attempt to clear nozzle clog by stream flush routines, with sort chamber door closed. If clog is not cleared, remove the nozzle and dependent upon sample risk assessment, decontaminate nozzle before sonication

3. Decontamination procedures

    a. Decontaminate and clean sample lines, sort chamber and collection chamber.
    b. Decontaminate and clean surfaces around cytometer, especially near the sort chamber

Development of the SOP should also include consultation with DOHS Safety and Health Specialist who can provide guidance on general biosafety procedures as well as information on NIH policy. Examples of SOPs for cell sorters are included in Appendix III to serve as

templates for development of individual laboratory SOPs. **Finally, the SOP should be reevaluated at least on an annual basis or whenever there is a change in instrument configuration that may affect biosafety.**

III. Specific Requirements for Operation of Cell Sorters in NIH Laboratories Housing for Cell Sorters

Biosafety Level 2 (BSL-2) Laboratory – General:

a. The laboratory must meet all criteria for BSL-2 containment and be surveyed and posted by DOHS.
b. Air flow in the room is balanced to create negative airflow into the room.
c. Laboratories must have a sink for hand washing. The sink may be operated manually, hands-free, or automatically. It should be located near the exit door.
d. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
e. Vacuum lines should be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
f. An eyewash station must be readily available.

Biosafety Level 2 (BSL-2) - With Enhanced Precautions:

a. Ideally, the cell sorter is located in a separate, lockable room where no other laboratory activity is performed. If the sorter is located in shared laboratory space, all Personal Protective Equipment (PPE) requirements (see G. Procedures IV - 4 ***User Specific Safety Equipment and Appendix I***) should be followed by all personnel during sorting procedures. The cell sorter should be placed in a location in the lab so that directional air flow is toward the cell sorter and away from other areas of the lab. If the cell sorter is enclosed within a certified BSC (Class I or Class II), the requirement for placement of the cell sorter in a separate room may be abrogated dependent upon the overall risk assessment.
b. Air flow in the room is balanced to create negative airflow into the room.
c. The sorting room is locked to restrict access to allow the operator to concentrate on the sort and to maintain regular air flow and negative air pressure in the room.
d. During sorting procedures, a sign should be placed on the outside of the door to indicate that a potentially biohazardous sorting process is in progress. This sign also should contain all necessary information for entering the room safely, including warning for Class IIIb or IV lasers, if applicable.

Biosafety Level 3 (BSL-3) – Laboratory:

a. The laboratory must meet all criteria for BSL-3 containment and be surveyed and posted by DOHS.
b. The cell sorter must be located within a Class II certified BSC (can be recirculated).

Biosafety Level 4 (BSL-4) Laboratory:

a. The laboratory must meet all criteria for BSL-4 containment and be surveyed and posted by DOHS.
b. The cell sorter must be located within a Class II or Class III certified BSC.

IV. Cell Sorter-Specific Safety Equipment and Practices

1. **Aerosol Containment:**

Aerosol management System (AMS): All cell sorters must be equipped with an aerosol management or evacuation system that is designed to evacuate the sort chamber and sort collection area of the cytometer. It consists of an evacuator that creates negative pressure within those chambers, and transports aerosols through a HEPA or an ultra-low penetration air (ULPA) filter before exhausting to the room. The AMS should be operated under all biosafety levels, BSL-2, BSL-2 with enhanced precautions, BSL-3, and BSL-4.

1. **Validation of Aerosol Management Systems:**

Currently, the most widely accepted method of containment testing utilizes fluorescent plastic beads that are run on the instrument as a sample (See references 3, 4 and 5 above). The AMS must be tested under simulated worse-case "failure mode." In this mode, the instrument is set to high pressure (usually 70psi), and fluorescent particles are concentrated to approach speeds of approximately 20,000-50,000 particles/second. The stream is forced to glance off of the waste catcher shield to create a large plume of aerosols and aerosols concentrated on a slide for subsequent analysis on a microscope. Tolerance of aerosol escape is zero particles when the AMS is active and sort chamber door is closed. This test (or other validated test for containment) is performed periodically (monthly or only when filters are exchanged) for BSL-2 labs and labs performing sorts under BSL-2 with enhanced precautions. The test is performed prior to every sort for BSL-3 labs. Frequency of testing will be dependent upon the risk assessment and consultation with biosafety professionals and/or the IBC. However, containment testing must be performed in the following circumstances:

a. Following instrument service or maintenance involving the sort chamber and/or AMS hose connections.
b. Following initial instrument installation or relocation.
c. Following change out of the standalone AMS filter.
d. For BSL-3 or 4 labs:

    i. Prior to every sort if the frequency of sorting is once/week or less
    ii. Weekly, if the frequency of sorting is multiple sorts/week

3. **Cell sorters in biological safety cabinets:**

Class II BSC's enclosing cell sorters must be manufactured to meet functional certification criteria for personnel and product protection as defined by NSF 49. Class I BSC's enclosing cell sorters must be manufactured to meet functional certification criteria for personnel protection as defined by the BMBL, although it is recommended that the inward airflow velocity be 100 linear feet per minute or greater; HEPA filters must be tested for leakage annually. Cell sorters placed in BSC's must have an AMS in which aerosol containment validation can be performed independent of the BSC blowers, i.e. with the BSC directional air current system turned off. This is done to provide greater sensitivity when performing the cell sorter AMS containment tests. The BSC must be validated initially at installation. Frequent retesting and monitoring proper functioning of the cabinet is mandatory, as per NSF 49 requirements.

4. **User Specific Safety Equipment:**

   a. **Personal Protective Equipment (PPE) for Biosafety Level 2 (BSL-2) Laboratory:**

      i. Front closure lab coat
      ii. Gloves
      iii. Eye Protection: Safety glasses

   b. **Personal Protective Equipment (PPE) for Biosafety Level 2 (BSL-2) with enhanced precautions:**

      i. Isolation-style solid-front or wrap-around gown
      ii. Gloves
      iii. **Eye protection:** Safety goggles face shield, splatter guard or integral respirator/face shield that provide mucous membrane protection as required for anticipated splashes or sprays of infectious agents or other hazardous materials.
      iv. **Respirator:** National Institute for Occupational Safety and Health (NIOSH)-approved respirators must be worn during operation of the cell sorter under BSL-2 with enhanced precautions conditions. Approved respirators include N-95, N-99, or N-100 filtering face-piece respirators or powered air-purifying respirators (PAPR) with integral face shield. 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. For non-primate samples containing agents that do not pose respiratory risk, mucous membrane protection may be substituted for respirators. For example, the human pathogens leishmania and mouse models of toxoplasma infection are included in this category.
      v. **Cell Sorters enclosed in a certified BSC:** use of respirators as outlined above is recommended during instrument/sample manipulation within the BSC but can otherwise be removed during sorting procedures providing the

BSC is operational, aerosol management system is active and all sort chamber and collection chamber doors are closed. If the BSC-enclosed Cell Sorter is in a shared laboratory, respirators are not required for other laboratory personnel.

vi. All individuals using respirators must be enrolled in the NIH Respiratory Protection Program. Supervisors are not authorized to select respiratory protective devices. Questions should be directed to the DOHS Technical Assistance Branch (TAB) at 301-496-2960

c. **Personal Protective Equipment (PPE) for Biosafety Level 3 (BSL-3) Laboratory:**

i. Liquid-resistant scrub suits or coveralls

ii. Gloves (double pair). Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Gloves and protective clothing must not be worn outside the laboratory and must be disposed of with other contaminated waste.

iii. **Eye Protection/Respirator:** NIOSH-approved powered air purifying respirators (PAPR) with integral face shield must be worn at all times when in the laboratory. If you have any questions, contact the DOHS Technical Assistance Branch at 301-496-2960. Eye and face protection must be disposed of with other contaminated waste or decontaminated before reuse.

d. **Disinfection:**

The choice of disinfectant is dependent upon a variety of factors (4) including the agent in use, the chemical resistance of the cell sorter components, and potential of exposure of lab personnel to the chemical disinfectant. Broad-spectrum disinfectants are desirable in a facility in which agent use is varied. Sodium hypochlorite solutions (1:10 dilution of household bleach in H_20; final concentration of 5,250-6,150 ppm of chlorine) offer several advantages over alcohols and other disinfectants; bleach has broad-spectrum antimicrobial activity, does not leave toxic residues, is unaffected by water hardness and is inexpensive and fast acting. However, because of the corrosive nature to metals, exposure to instrumentation should be limited to times determined to be maximally efficacious to microbial killing. In addition, bleach solutions must be prepared fresh due to loss of free available chlorine. However, there are commercially available sprayers that mix the bleach and water when sprayed, eliminating the need to make fresh solutions daily.

I. Records Retention and Disposal

All records pertaining to this chapter must be retained and disposed of under the authority of NIH Manual 1743,"Keeping and Destroying Records," Appendix 1, "NIH Records Control Schedules" (as amended). These records must be maintained in accordance with current NIH

Records Management and Federal guidelines. Contact your IC Records Liaison or the NIH Records Officer for additional information.

J. Internal Controls

The purpose of this Manual Chapter is to establish the NIH policy for the installation and operation of the class of laboratory instruments known as cell sorters, or fluorescent activated cell sorters, and is designed to protect worker safety, public health, and the environment.

1. **Office Responsible for Reviewing Management Controls Relative to this Chapter (Issuing Office)**: Through this manual issuance, the DOHS, ORS is accountable for the method used to ensure that management controls are implemented and working.
2. **Frequency of Review (in years):** Annual at a minimum; more often as necessary.
3. **Method of Review:** The DOHS will maintain oversight and ensure effective implementation and compliance with this policy. Annual reviews of the policy will be done in comparison to current trends in the field of flow cytometry and a review of NIH practices.
4. **Review Reports are sent to:** An annual summary will be sent to the Director, DOHS. Issues of concern will be brought to the attention of the Director, for the Office of Research Services (ORS).

Appendix I: Biosafety Level Determination for Cell Sorting

	BSL-2	**BSL-2 with enhanced precautions (during sorting operations)**	**BSL-3**	**BSL-4**
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**	**Extreme Dangerou Pathoge**
Example Sample type or Agents[1]	Normal murine cells 3rd generation Lentivirus (non-human cells)	Normal human blood Human cell lines[1] An example agent is: Influenza A[1] 2nd generation Lentivirus or 3rd generation in human cells	Example agents include[1]: Mycobacterium Tuberculosis, Monkeypox	Example agent include[1]: Ebola, Marburg
Containment System Validated	Periodically (monthly or with filter change)	Periodically (monthly or with filter change)	Before Every Sort	Before Every
Aerosol Containment Operational	Required	Required	Required	Require
Respirator	Optional	N-95 or better[3]	PAPR	Special S
Eye protection	Safety Glasses	Face shield or safety goggles	N/A	N/A
Lab Coat	Front Closure lab coat	Wrap around rear closure	Coveralls	Special su
Separate Room and Environmental controls	Optional	Required or limited access to room[4]	Required[5]	Require

[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; https://www.cdc.gov/biosafety/publications/bmbl5/index.htm**

[2]**Frequency of testing will be dependent upon the risk assessment and consultation with biosafety professionals and/or the IBC or equivalent. For more detail see Section H. Procedures: Cell Sorter-Specific Safety Equipment and Practices.**

[3]**. 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 with a containment recommendation of BSL-2 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.**

[4]**Enclosure of the cell sorter within a certified (Section F: Definitions and Section H. Procedures: Cell Sorter-Specific Safety Equipment and Practices) BSC may abrogate the need to house the sorter in a separate room within the BSL-2 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 BSL-2 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.**

[5]**Enclosure of cell sorter within a certified (Section F: Definitions and Section H. Procedures: Cell Sorter-Specific Safety Equipment and Practices) BSC required.**

Appendix II: Example Agent List with Biosafety Level for Cell Sorting

Agent	Recommended Biosafety Level	Restrictions or Comments	MSDS Link
Hepatitis C	BSL-2+[2]		http://www.phac-aspc.gc.ca/msds-ftss/msds77e-eng.php
Human Metapneumovirus	BSL-2+		
Human Parainfluenza	BSL-2+		

Agent	Recommended Biosafety Level	Restrictions or Comments	MSDS Link
Virus type 3			
Influenza A	BSL-2+	Influenza (seasonal) vaccine required	
Klebsiella pneumonia	BSL-2+		http://www.phac-aspc.gc.ca/msds-ftss/msds90e-eng.php
LaCrosse virus	BSL-2+		
LCMV	BSL-2+ or BSL-3	Ensure that HVAC system does not exhaust near vivarium housing mice; BSL dependent upon strain; pregnant women should consult Occupational Medical Service (OMS) or their personal physician prior to performing a procedure with this agent.	http://www.phac-aspc.gc.ca/msds-ftss/msds97e-eng.php
Leishmania	BSL-2+[3]		http://www.phac-aspc.gc.ca/msds-ftss/msds94e-eng.php
Malaria	BSL-2+[3]		
PVM (Pneumonia Virus of Mice)	BSL-2+		
Respiratory Syncytial Virus	BSL-2+		http://www.phac-aspc.gc.ca/msds-ftss/msds125e-eng.php
Toxoplasma gondii	BSL-2+	Pregnant women should consult OMS or their personal physician prior to performing a procedure with this agent.	http://www.phac-aspc.gc.ca/msds-ftss/msds153e-eng.php
Vaccinia	BSL-2+	vaccine required	http://www.phac-aspc.gc.ca/msds-ftss/msds160e-eng.php
1918 Influenza	BSL-3	Influenza (seasonal) vaccine required	
Avian influenza	BSL-3	Influenza (seasonal) vaccine required	
H1N1	BSL-2	H1N1 vaccine required;	

Agent	Recommended Biosafety Level	Restrictions or Comments	MSDS Link
HIV	BSL-2+ or BSL-3		http://www.phac-aspc.gc.ca/msds-ftss/msds84e-eng.php
Monkeypox	BSL-3	vaccine required, every 3 years	
TB, Mycobacterium tuberculosis	BSL-3		http://www.phac-aspc.gc.ca/msds-ftss/msds103e-eng.php

[1]This list represents examples of biosafety level determination for cell sorting of specific agents. The final determination of the biosafety level is dependent upon the risk assessment conducted in collaboration with DOHS Safety and Health Specialist , subject matter experts and the NIH IBC.

[2]BSL-2 with enhanced precautions is abbreviated BSL-2+ for this table.

[3]Respirator PPE optional (mucous membrane protection required) for this agent except where the sample also contains human/NHP blood cells or fluids.

Appendix III: Examples of Standard Operating Procedures

These can be used as guidelines for formulating a Standard Operating Procedure for individual laboratories. Procedures and practices will vary dependent upon risk assessment and instrument designs. Product or company names used in these examples do not in any way constitute explicit or implicit endorsement of these products or companies by the NIH.

BSL-2 SOP

1. Wear Lab Coat and Gloves
2. Turn on Aerosol Management System (biohazard vacuum) and operate at 20% or as recommended by instrument manufacturer

    a. Check vacuum reading. If vacuum is >2.4 inches of H_2O, change HEPA filter. Note: HEPA filter must be changed every 6 months, regardless of vacuum reading.
    b. Procedure for changing HEPA filter on AMS unit:

        i. While wearing gloves, lab coat, N-95 rated face mask (respirator) or PAPR and goggles/safety glasses, place the Buffalo unit HEPA filter inside an orange biohazard plastic bag. Disconnect hose from the Aria and also place within the bag. Seal the bag and place within a Medical Pathological Waste (MPW) box. Install a new HEPA filter and hose.

3. Make sure collection chamber door and sort chamber door are closed during sorting procedures
4. Do not eat or drink in laboratory
5. Remove gloves before answering phone
6. Remove lab coat and gloves and wash hands before leaving lab

BSL-2 with enhanced precautions SOP - FACS Aria II

1. **Preparation before the sort**

    a. If not using a sealed keyboard and mouse, cover keyboard, mouse and other instrument control surfaces w/ plastic wrap; clear surfaces of clutter, use absorbent pads for samples.
    b. Using a damp paper towel(s), wipe up dried bleach residue from instrument areas, paying particular attention to the sample uptake area, O-rings, charge plates and the side stream viewing window. Warning: Failure to remove salt residue from the sample uptake system may cause the pressurized seal to fail and release potential aerosols!
    c. Prepare sort collection chamber as necessary. Install the correct collection tube holder. Close sort collection chamber door.
    d. If the Aria is contained within a BSC, turn the BSC blower fan on and turn the evacuation vacuum on low.
    e. If not using a BSC, turn biohazard vacuum (Buffalo Filter Whisper Unit) on and operate at 20%. Check vacuum reading. If vacuum is >2.4 inches of H_2O, change HEPA filter. Note: HEPA filter must be changed every 6 months, regardless of vacuum reading.

        i. Procedure for changing HEPA filter on AMS unit:

            1. While wearing gloves, closed front lab coat, N-95 rated face mask (respirator) or PAPR and goggles/safety glasses, place the Buffalo unit HEPA filter inside an orange biohazard plastic bag. Disconnect hose from the Aria and also place within the bag. Seal the bag and place within a Medical Pathological Waste (MPW) box. Install a new HEPA filter and hose.

    f. Make sure sheath tank is filled and standard waste tank contains enough bleach to give a final 10% (1:10 dilution of household bleach) solution when filled. Fill a spray bottle with a freshly made 10% (1:10 dilution) bleach solution for work area decontamination.
    g. Wear gloves, lab coat, N-95 rated face mask (respirator) or PAPR and goggles/safety glasses (or N-95 mask with face shield) before handling samples.
    h. Lab door must be closed and investigators are to remain outside of the lab until data files of the experimental controls and samples have been collected and tubes are no longer being manipulated.

i. For areas within a BSC, wear gloves, closed front lab coat, N-95 rated face mask (respirator) or PAPR and goggles/safety glasses (or N-95 mask with face shield) before handling samples. Lab door may remain open, but notification of a potential biohazard must be posted outside the lab entrance. Investigators may remain in the room during data file collection.

j. 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 as outlined below in Sections 3. Note that respirator protection may otherwise be removed during the sorting process except during procedures as outlined above.

k. Have a spare nozzle, with new O-ring installed, available in case of a clog.

1. **Procedures during sorting/analysis**

a. Filter samples prior to sort to avoid clogs
b. Fill sample tube with as much sample as possible to minimize loading and unloading sample. DO NOT fill higher than ¼ inch from the top of the tube.
c. Make sure the "Sweet Spot" is enabled.
d. Close sort collection chamber door before starting sample.
e. When changing collection tubes:

1. Stop the sample flow and close the aspirator drawer by clicking the Acquire button.
2. Wait at least 60 seconds before opening sort collection chamber door.

f. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, use alcohol swab or bleach to wipe outside of tubes.

3. **Procedures in the event of a nozzle obstruction**

a. If during the sort the stream is deflected (due in part to a clogged nozzle), the sort is designed to stop automatically and block the sort tubes. The sort will not restart until the operator has cleared the clog. In the event of a nozzle clog , DO NOT open sort collection chamber door or sort block door before following this procedure:

i. If the system has not already shut down automatically, turn off the stream using the button labeled with an '✓' on the Breakoff window. This will shut off the stream, unload the sample and close the aspirator door. Remove and cap the sample tube.

1. With the sort block chamber door, aspirator drawer and collection chamber door all closed, turn the stream on and off several times or

perform the 'Clean flow Cell' procedure with DI H_20 followed by turning the stream on to see if the clog will clear itself.

ii. Turn stream off.
iii. Open aspirator drawer using software controls.
iv. Increase the air evacuation rate on the AMS unit to 100% or if using a BSC, push the high evacuation button (low button must also remain on).
v. Wait at least 60 seconds. This procedure will clear aerosols from the sort chamber. Close the aspirator drawer.
vi. The sort block chamber door and sort collection chamber door can now be opened.
vii. If it is necessary to change nozzles, remove nozzle and O-ring and place in tube with 10% (1:10 dilution) bleach for 30 minutes. Thoroughly rinse nozzle in water and let air-dry. Discard O-ring if not using nozzles with integrated O-rings. Spare integrated nozzle or spare nozzle with O-ring may be installed while obstructed nozzle is soaking in bleach.
viii. With stream turned off, open the sort block chamber door and dry plates and surfaces as needed.
ix. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, use alcohol swab or bleach to wipe outside of tubes.
x. Set AMS unit to 20% vacuum or toggle the high evacuation button off if using a BSC.
xi. Make sure that all chamber doors are closed and restart the stream.

4. **<u>Aerosol Release/Spill Response Procedures</u>**

a. In the event of an aerosol release or a spill of infectious sample outside of Biological Safety Cabinet, the following protocol must be followed.

i. Aerosol Release Definition: The engineering controls on the Aria (Sort Chamber door, Collection Chamber door and Aerosol Management system) and the SOP in this document are designed to prevent aerosol release into the room. Failure of these systems or failure to follow the SOP may result in an aerosol release. The most likely scenario for an aerosol release is opening the sort chamber door, during, or immediately following a nozzle obstruction.
ii. In the event of an aerosol release or spill of infectious material:

1. Push the Emergency Stop Button, and immediately exit the lab, closing the door as you leave. (All personnel must immediately exit the room)
2. Wait 30 minutes, and then do respirator, gloves and lab coat as detailed above.
3. Enter the lab and clean any spill using 10% bleach HypeWipe pads. Clean horizontal surfaces near the cell sorter, or near the spill

location using HypeWipe pads. Respirator may be removed after all cleaning procedures have been performed.

5. **Decontamination Procedures:**

a. Disengage "Sweet Spot" and turn the stream off.
b. Disinfect sample lines using a freshly made 10% bleach solution as follows:

i. Fill a tube with a volume of 10% bleach equal to or greater than the volume of sample that was sorted and place on the sample stage.
ii. Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell. Perform this step three times or until a bleach drop is visible in the stream camera view.
iii. Wait 30 or more minutes with 10% bleach in flow cell.
iv. Fill a tube with DI water, Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell.
v. Fill a tube with 70% ETOH, Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell. Perform this step three times or until an ETOH drop is visible in the stream camera view. Shutdown instrument.

c. Clean all surfaces around optical bench, sort block chamber and charge plates, sort collection chamber, sample introduction area and sample tube holder(s) with a prepackaged 10% bleach towel and/or 10% (1:10 dilution) bleach from a spray bottle. Clean keyboard cover, remove any plastic wrap that may have been used and discard in MPW box.
d. When leaving the lab:

i. Make sure all samples are capped.
ii. Remove gloves, respirator & lab coat (remember outside of gloves are contaminated!).
iii. WASH HANDS!

BSL-3 SOP - FACS Aria II

1. **FACS Aria Cell Sorter**

a. The FACS Aria Cell Sorter and associated Aerosol Management System are located within a Class II Biological Safety Cabinet with Dual door assembly. The hood doors must be closed with sash windows in the closed position and the hood operational during all procedures involving infectious agents.

b. Instrument Pre-Sort Check and Supplies Check must be performed as outlined in Section 3 below.

2. **Aerosol Management System (AMS)**

a. While sorting viable infectious material (infected cells) the following guidelines must be followed for proper aerosol containment. All sort operators in this section must be trained and certified by the Flow Cytometry Section prior to any cell sorting operations.
b. The AMS must be on and functioning according to the manufacturer guidelines. The vacuum control should be set to 20% and the vacuum gauge less than 2.4 inches of H_2O. If it is outside of this range, replace HEPA filter unit and tubing.
c. HEPA filter must be changed under the following conditions:

   i. The vacuum monitor gauge reads 2.4 inches of H_2O or greater at 20% suction.
   ii. Three months has passed since installation of the filter.

d. Care must be taken when removing filter and associated hose since these are assumed contaminated. After changing the hose and filter, ensure that the Filter Life Reset button has been pushed and the new filter has been dated.
e. The Accudrop camera system must be functioning normally according to the manufacturer guidelines. This camera system is used to monitor the sort stream and alerts the operator to potential increased aerosols. In this situation the operator can correct the sort stream and reduce aerosol contamination. The FACSAria is equipped with a Sweetspot which is used during all sorting operations. This device can detect stream drifts and possible clogs, and automatically shut downs the stream.

3. **Preparation before the sort: Instrument Pre-Sort Check and Supplies Check**

a. Bleed Fluidics Cart filters
b. Fill sheath tank. Empty waste if necessary by thoroughly mixing closed waste container, and disposing in sink followed by large amounts of water. Fill waste tank with 10% final bleach concentration. Prepare fresh 10% bleach solution daily.
c. Close BSC cabinet doors.
d. Turn on Instrument and launch software.
e. Using a damp paper towel(s), wipe up dried bleach residue from instrument areas, paying particular attention to the sample uptake area, O-rings, charge plates and the side stream viewing window. Warning: Failure to remove salt residue from the sample uptake system may cause the pressurized seal to fail and release potential aerosols.
f. Open sort block chamber door and verify that aspirator door is operational using software controls
g. Perform instrument Quality Control procedures.
h. Verify that all supplies are stocked.

4. **Procedures during infectious sort**

a. The flow cytometer must pass all tolerances of aerosol containment. If these tolerances are not met, infectious cell sorting is not permitted.
b. The Class II Biological Safety Cabinet must be turned on.
c. Turn on and verify that the AMS is working correctly. This device must have a vacuum pressure of <2.4 inches of H_2O. If this tolerance is not met, infectious cell sorting is not permitted.
d. Close all barriers around the sort chamber. If this is not done, infectious cell sorting is not permitted.
e. While within a Class II Biosafety cabinet, all samples for sorting must be filtered through a 50 µm mesh prior to sorting. This reduces the potential for clogging and decreases the risk of creating aerosols.
f. Place sample onto the sample station. Start sort and monitor the sort performance using the Accudrop camera.

5. **Procedures in the event of a nozzle obstruction**

a. If during the sort the stream is deflected (due in part to a clogged nozzle), the sort is designed to stop automatically and block the sort tubes. The sort will not restart until the operator has cleared the clog. In the event of a nozzle clog , DO NOT open sort collection chamber door or sort block door before following this procedure:

    i. If the system has not already shut down automatically, turn off the stream using the button labeled with an '✓' on the Breakoff window. This will shut off the stream, unload the sample and close the aspirator door.
    ii. Open aspirator drawer using software controls.
    iii. Increase the air evacuation rate on the AMS unit to 100%.
    iv. Wait at least 60 seconds. This procedure will clear aerosols from the sort chamber. (Note that this step assumes that a modification to tube holder(s) (universal top component on Aria II) involving the drilling of 3 holes and the sort chamber door involving the drilling of 1 hole with attachment of 0.22µm filter, has been previously performed. (1))
    v. Close the aspirator drawer.
    vi. With the sort block chamber door, aspirator drawer and collection chamber door all closed, turn the stream on and off several times or perform the 'Clean flow Cell' procedure with DI H20 followed by turning the stream on to see if the clog will clear itself.
    vii. Turn stream off.
    viii. Open the aspirator drawer and evacuate for at least 60 seconds before closing the aspirator drawer again.
    ix. The sort block chamber door and sort collection chamber door can now be opened.
    x. If it is necessary to change nozzles, remove nozzle and O-ring and place in tube with 10% (1:10 dilution) bleach for 30 minutes. Thoroughly rinse

nozzle in water and let air-dry. Discard O-ring if not using nozzles with integrated O-rings. Spare integrated nozzle or spare nozzle with O-ring may be installed while obstructed nozzle is soaking in bleach.

xi. With stream turned off, open the sort block chamber door and dry plates and surfaces as needed.

xii. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, use alcohol swab or bleach to wipe outside of tubes.

xiii. Set AMS unit to 20% vacuum.

xiv. Make sure that all chamber doors are closed and restart the stream.

6. **Decontamination Procedures:**

a. Disengage "Sweet Spot" and turn the stream off.

b. Disinfect sample lines using a freshly made 10% bleach solution as follows:

i. Fill a tube with a volume of 10% bleach equal to or greater than the volume of sample that was sorted and place on the sample stage.

ii. Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell. Perform this step three times or until a bleach drop is visible in the stream camera view.

iii. Wait 30 or more minutes with 10% bleach in flow cell.

iv. Fill a tube with DI water, Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell.

v. Fill a tube with 70% ETOH, Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell. Perform this step three times or until an ETOH drop is visible in the stream camera view.

vi. Replace integrated nozzle with closed loop nozzle.

vii. Perform Instrument Shutdown as prompted in software; use 70% EtOH for cleaning solution. Place sample tube holder into tube containing 10% bleach for 30 minutes. Wash tube holder and cam in DI water and let air dry.

viii. Remove sheath probe and drain residual sheath from probe. Place probe on absorbent paper in hood. Empty sheath tank and place in bag for autoclaving.

ix. Turn off AMS. Verify that vacuum gauge is at zero.

x. Clean all surfaces around optical bench, sort block chamber and charge plates, sort collection chamber, sample introduction area and sample tube holder(s) with a prepackaged 10% bleach towel and/or 10% bleach from a spray bottle.