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| Title: **OEHS Standard Operating Procedure:**  **Working With Cell Sorters** | | Print Date:  **3/18/2019** |
| Revision # | Update Prepared By:  Neil E. Bowles | Draft Prepared:  **03.04.2019** |
| Effective Date: | Reviewed By:  Derek Hedquist  James Stubbs  David Gillespie  Debbie Eckert | Date Reviewed: |
| Standard:  BMBL,  NIH  International Society for the Advancement of Cytometry Cell Sorter Biosafety Standards | Approved By:  James Stubbs | Date Approved: |
| Revision History | A – Inception 2019 |  |

**Procedure**

# 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 OEHS Standard Operating Procedure 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.

# Scope

The objectives and responsibilities set forth in this SOP are applicable to all University of Utah employees. University of Utah employees will comply with this policy and perform their duties in the safest possible manner.

# Background

Flow cytometric cell sorting is 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.

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) at high pressure (up to 70psi) impacting a hard surface.1 Aerosol production is highest in the event of a partial obstruction of the nozzle orifice and subsequent stream deviation.

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 and adapted from established biosafety principles as outlined in the [*Biosafety in Microbiological and Biomedical Laboratories (BMBL)*](https://www.cdc.gov/labs/BMBL.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fbiosafety%2Fpublications%2Fbmbl5%2Findex.htm)*, current edition*, the [*National Institutes of Health*](https://policymanual.nih.gov/3038) and the current [*International Society for the Advancement of Cytometry (ISAC) Cell Sorter Biosafety Standards*.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4117398/)

# 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 SOP 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, safety glasses, facemasks, 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 or 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. **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](http://standards.nsf.org/apps/group_public/download.php/3604/NSF_49-08e-rep-watermarked.pdf); Class I BSC: manufactured to meet functional certification criteria for personnel protection as defined by the [BMBL](https://www.cdc.gov/labs/BMBL.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fbiosafety%2Fpublications%2Fbmbl5%2Findex.htm) 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.
8. **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).
9. **Institutional Biosafety Committee (IBC)** - The Institutional Biosafety Committee reports to the Vice President of Research or his or her designee on matters pertaining to the control of biological hazards. The 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 at the University of Utah.
10. **Human Pathogens** - Human Pathogens are agents (such as viruses, bacteria, prions, or fungi) that cause disease in humans.
11. **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.
12. **Agent Risk Group**-The classification of an infectious microorganism 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](https://www.cdc.gov/labs/BMBL.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fbiosafety%2Fpublications%2Fbmbl5%2Findex.htm), ranging from least likely to cause human disease (Risk Group 1) to highly likely to cause serious or lethal disease (Risk Group 4).

# Roles and Responsibilities:

## Principal Investigators (PIs)/Facility Directors:

* + 1. Must disclose the plan to work with Cell Sorters to the IBC, by completion of an IBC registration in BioRAFT.
    2. Ensure compliance from all personnel that work with Cell Sorters with all provisions of this SOP and any other special requirements or procedures specific to the facility within which they are working.
    3. Develop and review annually a laboratory-specific SOP for the Cell Sorting laboratory in compliance with this SOP. **The SOPs must be reevaluated at least on an annual basis or whenever there is a change in instrument configuration that may affect biosafety.**
    4. Incorporate the detailed SOP into their laboratory Biosafety Manual and provide a copy to the IBC.
    5. Ensure that all staff members are trained on, and follow the procedures established in this document and laboratory Biosafety Manual. Documentation of required training components (such as Bloodborne Pathogens Training, Biosafety Level 2 training, etc), as well as laboratory-specific training, must be provided to the IBC prior to approval

## Occupational and Environmental Health and Safety (OEHS):

* + - 1. Provide periodic review of this SOP and its application as needed.
      2. Provide guidance to PIs and the IBC in assessment of risk, including the determination of biosafety level appropriate for the type of samples and/or agents that will be sorted, and the appropriateness of engineering and work practice controls, when necessary.
      3. 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, provide information on medical clearance and perform fit-testing, if necessary.
      4. Provide technical consultation for respiratory protection needs, including written plan assistance and fit-testing services.
      5. Conduct audits of the laboratory where the procedures are conducted prior to IBC approval and annually thereafter.

## Institutional Biosafety Committee (IBC)

* + - 1. Review and approve the biological materials registration for the use of the Cell Sorter.

## Employees

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

# Procedures:

## Risk Assessment

A risk assessment should be conducted for all samples/agents prior to sorting by the Principal Investigator/Facility Director, and the appropriate biosafety level determined in collaboration with OEHS Biosafety Specialists, subject matter experts, and the Institutional Biosafety Committee. 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: To aid in the identification of risks associated with biohazardous agents, microbiological agents have been classified into one of four Risk Groups (RG) by the WHO, Canada, Australia, the European Union and the NIH Recombinant DNA Advisory Committee and the American Biosafety Association ([ABSA](https://my.absa.org/tiki-index.php?page=Riskgroups)). Although these classifications differ dependent upon the country or organization, they generally will take into account factors such as pathogenicity of the organism, virulence, mode of transmission, infectious dose, communicability and availability of effective vaccines or effective treatment. There is a wide range of risk within each risk group classification, underscoring the importance of conducting a risk assessment for each biohazard. Agent characteristics to consider may involve the degree of attenuation, fixatives used to inactivate the agent, route of infection, and how a pathogen may have been rendered defective. Other characteristics that could elevate risk include the use of strains for which immunization is not protective, prior LAIs with the agent via the airborne route, and agents with a high consequence of infection.

1. Identify laboratory procedure hazards: It is important to emphasize that the second major factor to consider in risk assessment are the laboratory procedures in agent handling. Procedures with biohazards involving the use of sharps, those involving research animals, and those that may generate splash, splatter or aerosols can elevate risk. For example, human pathogens that are designated as Risk Group 2 agents under normal laboratory procedures and practices may be classified at a higher biosafety containment level because of the potential for aerosol and/or splash exposure. Cell sorting is therefore considered a laboratory procedure hazard due its potential for aerosol production.
2. Make final determination of biosafety level (BSL) (See Appendix I): BSL2 with enhanced precautions is not a biosafety level, but reflects procedures and practices at the BSL2 level together with additional procedures as specified in Appendix I. The guidelines for risk assessment in Appendix I take into account both agent hazards and sample origins for assignment of biosafety containment levels and procedures. This therefore combines agent Risk Group classifications, coupled with the recognition of cell sorting as a laboratory procedure hazard. In this regard, handling of all human and non-human primate specimens and primary human cell cultures as infectious is recommended, unless comprehensive pathogen screening has been performed and demonstrated the absence of adventitious agents. Although impractical for most cell sorting experiments, samples may be fixed in order to reduce the biocontainment level required. However, in this case, appropriate methods must be selected to reliably inactivate potentially biohazardous agents. Concerns exist about the effectiveness of standard fixation methods to reduce the level of infectivity in samples containing high titers of known viruses or unknown infectious agents resistant to inactivation. Fixation procedures must be performed carefully within well-defined standard operating procedures; otherwise, samples that are presumed inactivated, may not be and therefore could pose a serious health risk to laboratory personnel. Cell sorting operators or managers and IBCs may require proof of inactivation for higher risk biohazards to ensure that the biosafety level selected is appropriate for the proposed sorting experiment.
3. Evaluate proficiencies of staff and integrity of safety equipment: It is critical to evaluate the level of proficiency of the cell sorter operator in conducting a risk assessment. This includes an evaluation of cell sorter operating skills, as well as techniques for safe handling of specimens and use of any safety equipment. Proficiency in the operation of the cell sorter is particularly important in the event of a nozzle obstruction with subsequent aerosol production. In this case, an inexperienced operator will focus on instrument operation and thus will be more likely to ignore or circumvent biosafety features and procedures resulting in potential exposure. Training of cell sorter operators is therefore an essential component of the cell sorting laboratory’s operational procedures. The amount of training deemed sufficient for independent operation of a cell sorter is dependent upon several factors, but must include the results of the risk assessment process. Specifically, for sorts requiring higher biosafety containment levels (BSL2 with enhanced precautions or BSL3) the degree of training and experience must be correspondingly greater. For independent operation of cell sorters at these biosafety containment levels, a checklist of requirements of experience/training is essential to ensure safe operation. These must include required institutional biosafety training such as bloodborne pathogen training, BSL2-specific training, BSC training, etc., but also instrument experience, such as hours of supervised and independent cell sorting operation. Ideally, before sorting samples at a higher biosafety containment level, initial training should include sorting on cell sorters of similar design using non-infectious samples of the same type that will contain the known biohazard. In addition, when procedures are changed, all operators should be required to review these procedures and documentation of this review must be maintained. All safety equipment must be inspected or tested to verify functionality. For cell sorters, evaluation of safety equipment includes visual inspection of sort/collection chamber doors to ensure integrity, or absence of dirt and/or salt crystals on seals; presence of an aerosol management system and validation of containment (see below) and verification that all other supplied safety features are intact.
4. Review risk assessment with OEHS Biosafety Specialists and the 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)](https://www.cdc.gov/labs/BMBL.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fbiosafety%2Fpublications%2Fbmbl5%2Findex.htm). Cell sorting is considered a laboratory procedure hazard because of the potential for aerosol and/or splash 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.

## 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
   1. Check fluids, empty waste
   2. Cover control surfaces with plastic wrap, including keyboards and mouse (or use washable keyboards).
   3. Perform containment testing. A detailed procedures can be found [here](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4117398/#APP1)
2. Validation of containment and evacuation of aerosols is essential for operator safety. Testing on individual cell sorters may differ due to variation in cell sorter design among the available models, but it is essential that the following considerations are incorporated into the SOP:
   1. Fail mode testing: test is designed to mimic a nozzle obstruction with stream deviation and the subsequent generation of aerosols.
   2. Testing frequency: dependent upon risk assessment, and biosafety containment level.
   3. Containment testing for sorter in BSC: aerosol containment and evacuation of sorter independent of BSC operation must be performed.
   4. Record keeping.
   5. Verify sorting operation and sample introduction system
3. Before each sort, verify the proper operation of the sort mechanism and the stability of the sort streams and droplet break-off. If the streams and the droplet break-off do not remain stable during the sort setup, correct the problem before sorting.
4. Cell sorters pressurize the sample tube once it is secured on the sample introduction port. While newer generation instruments are equipped with completely enclosed sample introduction chambers for operator safety, some older sorters have an open port requiring careful operator handling. Each time a sample tube is placed on the instrument, the operator must check the tube seal and its secure fit onto the sample introduction port. Otherwise, once the sample tube is pressurized, it could blow off and splash sample onto the operator or others involved in the experiment. Make sure that the tube material provides sufficient strength to tolerate high instrument pressure. On some instruments, when the tube is removed, the sample line back-drips, creating a potential biohazard through splattering of sample droplets on hard surfaces. To avoid this hazard, allow the back-drip to go into a tube until the sample is flushed out of its introduction line to avoid splashing of sample droplets. Alternatively, a soft absorbent pad soaked in disinfectant can collect the back drip without splattering. Installation of a plastic shield around the sample introduction port can block droplet spraying from the sample back-drip. The catch tray or trough should be decontaminated carefully after each sort.
5. Select the appropriate nozzle size for the cell size to be sorted. Smaller nozzle sizes provide optimal signal resolution and easy sort setup, however, to avoid clogs, it is recommended that the nozzle orifice be at least four times larger than the cell diameter, but ideally it should be at least six times larger.
   1. Verify any automated decontamination functions
      1. If these systems are not used on a regular basis, it is possible that valves, connectors or pumps may fail due to buildup of salts, etc.
   2. Preparation of disinfectant solutions
      1. Disinfectants should be made before starting the sort, especially for those that have limited shelf life, such as solutions of sodium hypochlorite.
   3. 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
6. Procedures in the event of a nozzle obstruction
7. Turn off stream
8. Evacuate sort chamber prior to opening; increase Aerosol Management System (AMS) evacuation rate
9. 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
10. Decontamination procedures
11. All decontamination procedures should be validated and documented per [Institution Guidelines](https://d2vxd53ymoe6ju.cloudfront.net/wp-content/uploads/sites/4/20180824082203/Chemical-Disinfection-Fact-Sheet.pdf).
12. Decontaminate and clean sample lines, sort chamber and collection chamber.
13. Decontaminate and clean surfaces around cytometer, especially near the sort chamber after each sort, the instrument should be decontaminated with a disinfecting agent, taking into account the biohazards under study. Sort collection tube holders are heavily exposed to sample droplets and must be carefully decontaminated before handling. Before designing a cell sorter-specific decontamination protocol, the operator or laboratory manager should consult the instrument manufacturer for compatible disinfectants and refer to more complete resources for decontamination. Two disinfectants commonly used in cell sorters are alcohols and bleach. Alcohols are not classified as high-level disinfectants, because they cannot inactivate bacterial spores and penetrate protein-rich materials, and isopropanol is not able to kill hydrophilic viruses. Aqueous solutions of sodium hypochlorite are widely used because they have a broad spectrum of antimicrobial activity, are inexpensive, fast acting, are unaffected by water hardness and do not leave a toxic residue. They can be corrosive to metals and therefore should be rinsed with water following decontamination. All surfaces inside the sort chamber, the sample introduction port and holder, are wiped down with appropriate disinfectant. Disinfectant is also run through the instrument for the appropriate exposure time and then followed with distilled water to completely remove the disinfectant as some disinfectants are corrosive to instrument components (consult manufacturer), and residual disinfectant solution can affect the viability of sorted samples. Make sure that the water used for removal of the disinfectant is sterile and does not introduce new contaminants into the instrument.

Development of the SOP should also include consultation with OEHS Biosafety Specialists who can provide guidance on general biosafety procedures as well as information on University of Utah policy. Examples of SOPs for cell sorters are included in Appendix C 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.**

## Specific Requirements for Operation of Cell Sorters in University of Utah Laboratories

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

1. The laboratory must meet all criteria for BSL-2 containment and be surveyed and posted by OEHS: criteria are described in detail in the [University of Utah Biosafety Manual](https://oehs.utah.edu/resources/university-of-utah-biosafety-manual).
2. Air flow in the room is balanced to create negative airflow into the room. The door must remain closed at all times.
3. 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.
4. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
5. Vacuum lines should be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
6. An eyewash station must be readily available.

**Biosafety Level 2 (BSL-2) Laboratory – With Enhanced protections:**

1. 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 should be followed by all personnel in the laboratory (NOT just those using the sorter) 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.
2. Air flow in the room is balanced to create negative airflow into the room. The door must remain closed at all times.
3. 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.
4. 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:**

1. The laboratory must meet all criteria for BSL-3 containment and be surveyed and posted by OEHS: criteria are described in detail in the [University of Utah Biosafety Manual](https://oehs.utah.edu/resources/university-of-utah-biosafety-manual).
2. The cell sorter must be located within a Class II certified BSC (can be recirculated).

**There are no Cell Sorters in BSL-3 Laboratories at the University of Utah presently.**

**Biosafety Level 4 (BSL-4) Laboratory:**

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

**There are no BSL-4 Laboratories at the University of Utah presently.**

## Cell Sorter-Specific 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 2-4 below).

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:

* 1. Following instrument service or maintenance involving the sort chamber and/or AMS hose connections.
  2. Following initial instrument installation or relocation.
  3. Following change out of the standalone AMS filter.
  4. For BSL-3 or 4 labs:
     1. Prior to every sort if the frequency of sorting is once/week or less
     2. 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](http://standards.nsf.org/apps/group_public/download.php/3604/NSF_49-08e-rep-watermarked.pdf). Class I BSC’s enclosing cell sorters must be manufactured to meet functional certification criteria for personnel protection as defined by the [BMBL](http://www.cdc.gov/biosafety/publications/bmbl5/), 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](http://standards.nsf.org/apps/group_public/download.php/3604/NSF_49-08e-rep-watermarked.pdf) requirements.

**4. User Specific Safety Equipment:**

* 1. **Personal Protective Equipment (PPE) for Biosafety Level 2 (BSL-2) Laboratory:**
     1. **Lab coat**
     2. **Gloves**
     3. **Eye Protection**: Safety glasses (Impact resistant and side protection)
  2. **Personal Protective Equipment (PPE) for Biosafety Level 2 (BSL-2) with enhanced precautions:**
     1. **Isolation-style solid-front or wrap-around gown**, with cuffed sleeves or disposable sleeve covers
     2. **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.
     3. **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.
     4. **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.
        + All individuals using respirators must be enrolled in the University of Utah Respiratory Protection Program. Questions should be directed to OEHS at 381-581-6590
     5. **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.
  3. **Disinfection:**

The choice of disinfectant is dependent upon a variety of factors 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. For work involving human or non-human primate cell lines it must be an [EPA-Registered disinfectant](https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants).

Sodium hypochlorite solutions (1:10 dilution of household bleach in H2O; 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.

# 7. References

1. [Holmes KL. Characterization of aerosols produced by cell sorters and evaluation of containment. Cytometry A 2011:79;1000-8.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3319021/)
2. [Holmes KL, Fontes B, Hogarth P, Konz, R, Monard S, Peltcher CH, Wadley RB, Schmid I, Perfetto S. International Society for Advancement of Cytometry Cell Sorter Biosafety Standards. Cytometry A 2014:85;434-53.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4117398/#__ffn_sectitle)
3. [Oberyszyn AS. Method for visualizing aerosol contamination in flow sorters. Curr Protoc Cytom 2002: Chapter 3; Unit 3.5](https://www.researchgate.net/publication/23236923_Method_for_Visualizing_Aerosol_Contamination_in_Flow_Sorters)
4. [Perfetto SP, Ambrozak DR, Koup RA, Roederer M. Measuring containment of viable infectious cell sorting in high velocity cell sorters. Cytometry A 2003:52;122-30.](https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.10033)

**Appendix I: Biosafety Level Determination for Cell Sorting**

|  |  |  |
| --- | --- | --- |
|  | ****BSL-2**** | ****BSL-2 with enhanced precautions (during sorting operations)**** |
| ****Risk Assessment Condition**** | **Pathogen-free Human /NHP cells** **Uninfected non-primate cells** | **Unscreened Human /NHP cells**  **Infectious but with low risk assessment (e.g. Risk Group 2)** |
| ****Example: Sample type or Agents1**** | Normal murine cells  3rd generation Lentivirus transduced cells (non-human cells or pathogen-free human cells: >72 hours post transduction) | Normal human blood  Unscreened Human cell lines**1**  An example agent is: Influenza A**1** 2nd generation Lentivirus transduced cells or 3rd generation lentivirus in unscreened human cells |
| ****Containment System Validated**** | Periodically (monthly or with filter change) | Periodically (monthly or with filter change) |
| ****Aerosol Containment Operational**** | Required | Required |
| ****Respirator**** | Optional | N-95 or better2 |
| ****Eye protection**** | Safety Glasses | Safety Glasses or goggles plus face shield or mask |
| ****Lab Coat**** | Lab coat | Wrap around rear closure, cuffed or disposable sleeves |
| ****Separate Room and Environmental controls**** | Optional | Required or limited access to room3 |

1Example: 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 Biosafety specialists, subject matter experts and the IBC. For additional information please consult the following web sites: [http://www.phac-](http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php) [aspc.gc.ca/msds-ftss/index-eng.php](http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php); <https://www.cdc.gov/biosafety/publications/bmbl5/index.htm>

2Respirators 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.

3Enclosure of the cell sorter within a certified 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.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Agent** | **Recommended Biosafety Level1** | **Restrictions or Comments** | **MSDS Link** |
| Hepatitis C | BSL-2+**2** |  | https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/hepatitis-c-virus.html |
| Human Metapneumovirus | BSL-2+ |  |  |
| Human Parainfluenza Virus type 3 | BSL-2+ |  | <https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/human-parainfluenza-virus.html> |
| Influenza A | BSL-2+ | Influenza (seasonal) vaccine required | <https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/influenza-virus-type-a.html> |
| Klebsiella pneumonia | BSL-2+ |  | https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/klebsiella.html |
| 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. | https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/lymphocytic-choriomeningitis-virus.html |
| Leishmania | BSL-2+**3** |  | https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/leishmania.html |
| Malaria | BSL-2+**3** |  |  |
| PVM (Pneumonia Virus of Mice) | BSL-2+ |  |  |
| Respiratory Syncytial Virus | BSL-2+ |  | https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/respiratory-syncytial-virus.html |
| *Toxoplasma gondii* | BSL-2+ | Pregnant women should consult OMS or their personal physician prior to performing a procedure with this agent. | https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/toxoplasma-gondii-pathogen-safety-data-sheet.html |
| Vaccinia | BSL-2+ | vaccine required | https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/vaccinia-virus.html |
| HIV | BSL-2+ or BSL-3 |  | https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/human-immunodeficiency-virus.html |

1This 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 OEHS Biosafety, subject matter specialists and the University of Utah IBC.

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

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

The following is a list of agents that may require BSL-3 containment for sorting. Currently no such facilities are available at the University of Utah:

|  |  |  |  |
| --- | --- | --- | --- |
| **Agent** | **Recommended Biosafety Level** | **Restrictions or Comments** | **MSDS Link** |
| 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. | https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/lymphocytic-choriomeningitis-virus.html |
| 1918 Influenza | BSL-3 | Influenza (seasonal) vaccine required |  |
| Avian influenza | BSL-3 | Influenza (seasonal) vaccine required |  |
| HIV | BSL-2+ or BSL-3 |  | https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/human-immunodeficiency-virus.html |
| Monkeypox | BSL-3 | Vaccine required, every 3 years |  |
| TB, *Mycobacterium tuberculosis* | BSL-3 |  | https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/mycobacterium-tuberculosis-complex.html |

**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 University of Utah.

**BSL-2 SOP**

1. Wear Lab Coat, Eye Protection and Gloves
2. Turn on Aerosol Management System (biohazard vacuum) and operate at 20% or as recommended by instrument manufacturer:
   1. Check vacuum reading. If vacuum is >2.4 inches of H2O, change HEPA filter. Note: HEPA filter must be changed every 6 months, regardless of vacuum reading.
   2. Procedure for changing HEPA filter on AMS unit:
      1. 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**
   1. 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.
   2. 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!
   3. Prepare sort collection chamber as necessary. Install the correct collection tube holder. Close sort collection chamber door.
   4. If the Aria is contained within a BSC, turn the BSC blower fan on and turn the evacuation vacuum on low.
   5. 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 H2O, change HEPA filter. Note: HEPA filter must be changed every 6 months, regardless of vacuum reading.
      1. Procedure for changing HEPA filter on AMS unit:
         * 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 Biohazardous waste container. Install a new HEPA filter and hose.
   6. 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 1:10 dilution of bleach solution for work area decontamination.
   7. Wear two pairs of gloves, closed front lab coat, N-95 rated face mask (respirator) and goggles/safety glasses or PAPR before handling samples.
   8. 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.
   9. For areas within a BSC, wear gloves, closed front lab coat, and goggles/safety glasses (and N-95 mask, optional) before handling samples. Notification of a potential biohazard must be posted outside the lab entrance. Investigators may remain in the room during data file collection.
   10. Respirators must remain on during all procedures outside of the BSC 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. Note that respirator protection may otherwise be removed during the sorting process except during procedures as outlined above.
   11. Have a spare nozzle, with new O-ring installed, available in case of a clog.
2. **Procedures during sorting/analysis**
   1. Filter samples prior to sort to avoid clogs
   2. 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.
   3. Make sure the “Sweet Spot” is enabled.
   4. Close sort collection chamber door before starting sample.
   5. 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.
   6. 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**
   1. 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:
      1. 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.

* 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.
  + 1. Turn stream off.
    2. Open aspirator drawer using software controls.
    3. 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).
    4. Wait at least 60 seconds. This procedure will clear aerosols from the sort chamber. Close the aspirator drawer.
    5. The sort block chamber door and sort collection chamber door can now be opened.
    6. If it is necessary to change nozzles, remove nozzle and O-ring and place in tube with a 1:10 dilution of 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.
    7. With stream turned off, open the sort block chamber door and dry plates and surfaces as needed.
    8. 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.
    9. Set AMS unit to 20% vacuum or toggle the high evacuation button off if using a BSC.
    10. Make sure that all chamber doors are closed and restart the stream.

1. **Aerosol Release/Spill Response Procedures**
   1. In the event of an aerosol release or a spill of infectious sample outside of Biological Safety Cabinet, the following protocol must be followed.
      1. 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.
      2. In the event of an aerosol release or spill of infectious material:
2. Push the Emergency Stop Button, and immediately exit the lab, closing the door as you leave. (All personnel must immediately exit the room)
3. Wait 30 minutes, and then put on respirator, gloves and lab coat as detailed above.
4. 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:**
   1. Disengage “Sweet Spot” and turn the stream off.
   2. Disinfect sample lines using a freshly made 1:10 dilution of bleach solution as follows:
      1. Fill a tube with a volume of 1:10 diluted bleach equal to or greater than the volume of sample that was sorted and place on the sample stage.
      2. 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.
      3. Wait 30 or more minutes with 10% bleach in flow cell.
      4. Fill a tube with DI water, Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell.
      5. 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.
   3. 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 wipe and/or 1:10 dilution of bleach from a spray bottle. Clean keyboard cover, remove any plastic wrap that may have been used and discard in MPW box.
   4. When leaving the lab:
      1. Make sure all sample tubes and containers are closed.
      2. Remove gloves, respirator & lab coat (remember outside of gloves are contaminated!).
      3. WASH HANDS!