

**TITLE:****SAFE USE AND OPERATION OF HIGH-SPEED CELL SORTERS GUIDELINES**

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## 1.0 Introduction

### 1.1 Purpose

To establish minimum standards for the safe operation of high-speed cell sorters at Emory University.

### 1.2 Scope

These guidelines apply to all high-speed cell sorters at Emory University.

## 2.0 Guiding Principles

- High speed cell sorting is conducted using a stream-in-air cell sorter, operated by trained personnel deemed competent to run the equipment.
- Stream-in-air cell sorters are located in a laboratory with a Biosafety Level 2 (BSL-2) or higher designation. Whenever possible, stream-in-air cell sorters are housed under negative air-pressure in a room separate from the main laboratory area, or in a biosafety cabinet.
- Access to the cell sorter, and area housing the cell sorter is limited to approved personnel.
- Cell sorters are equipped with aerosol management systems (AMS).
- Engineering controls, training, and Personal Protective Equipment (PPE) requirements for the use of a stream-in-air cell sorter are dependent on the facility, type of sample, species, and pathogens.
- Each requested sort must have an approved biosafety protocol associated with it, which includes a risk assessment for sorting the specimen/agent(s).
- The Investigator requesting the sort is required to provide confirmation of biosafety approval to the cell sorting operator or facility manager.
- EHSO will oversee and assist with risk assessment.
- Refer to Table 1.0 for roles and responsibilities of involved personnel.

## 3.0 Equipment Specific Safety Practices

### 3.1 Aerosol Management System (AMS)

- The AMS is designed to evacuate the sort collection area and sort chamber of the cytometer. It transports aerosols through a HEPA or ULPA filter and then exhausts them to the room.
- The AMS must be validated at regular and pre-determined intervals.
  - Validation of the AMS is the responsibility of the operator or manager of the cell sorting facility.
  - Tolerance of aerosol escape is zero particles when the AMS is active and the sort chamber door is closed.
  - Validation is conducted according to manufacturer recommendations.
  - Validation is documented; records are kept in the area of the cell sorter and are readily available.
- The filter on the AMS must be changed at a minimum of every 6 months.
  - PPE, including a respirator, is worn during filter changes.
  - Used filters are discarded as biohazardous waste.
- The AMS is operated according to manufacturer's guidelines during the sort.

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## 4.0 Risk Assessment

- Refer to Table 2.0 for general guidelines for determining the BSL and general procedures for cell sorting.
- Table 3.0 lists examples of specific agents and Biosafety levels.
- Comprehensive risk assessments are often completed in collaboration with specialists, subject matter experts, the Biosafety Officer, and the Emory University Institutional Health and Biosafety Committee (IHBC). See Table 4.0 for a sample risk assessment guideline.
  - Risk assessments identify risk associated with the sort. Most risk can be determined by the risk groups and the Biosafety in Microbiological and Biomedical Laboratories (BMBL).

## 5.0 Personal Protective Equipment

- PPE requirements depend on the BSL designation of the laboratory in which the cell sorter is located. BSL-2 PPE is the minimum standard.
- Operators and other personnel in the area of the cell sorter wear a respirator during the sorting process and for 30 minutes after the end of sort/machine shut down.
  - Respirators are NIOSH approved filtering respirators, for example an N-95.
  - Alternatively, a powered air purifying respirator (PAPR) may be worn.
  - Personnel wearing respirators must be enrolled in Emory University's Respiratory Protection Program. This includes annual fit-testing, medical clearance exam, and training (Respiratory Protection Program).

## 6.0 Cell Sorting Procedures

- Surfaces in the cell sorting facility are kept clean and clear of clutter.
- The laboratory door, collection chamber door, and sort chamber door remain closed during cell sorting procedures. If the cell sorter is located inside a Biological Safety Cabinet, sash doors are lowered to the appropriate level according to manufacturer's recommendations, and the hood is operational during procedures.
- Samples are filtered prior to sorting to avoid clogs.
- A spare nozzle and/or O-ring are available in the event of a clog.
- Sample tubes are filled with as much sample as possible, but not higher than ¼" from the top of the tube, to minimize loading and unloading of samples.
- When changing tubes, 60 seconds elapses before opening the sample chamber door.
- Following sort, tubes are wiped with an alcohol swab or bleach, as the outside of the tube is potentially contaminated.
- In the event of a nozzle obstruction:
  - If the system has not already shut down automatically, the stream is turned off.
  - The air evacuation rate on the AMS unit is increased to 100%.
  - Attempts are made to clear the clog.
  - If necessary, the nozzle is replaced.
  - Collection tubes are not removed from the sort collection chamber until

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the sample acquisition has been stopped for at least 60 seconds.

## 7.0 Transporting of Samples

- Samples are transported in an appropriately labeled leak-proof container.
- If the sample contains biohazardous material, the container must be affixed with a biohazardous material sticker.
- Samples are contained in screw-top tubes, capped tightly.

## 8.0 Disinfection of Equipment

- The choice of disinfectant depends on the agent in use, the species of the sample, and potential exposure of lab personnel.
- A broad-spectrum disinfectant is preferred where agent use is varied.
- The instrument is decontaminated with an appropriate disinfectant. A bleach solution may be used to disinfect sample lines.
- Surfaces in the cell sorting facility are disinfected after completion of the sort.

## 9.0 Exiting Cell Sorting Facility

- Refer to department specific policies and procedures.
- The AMS is turned off; vacuum gauge is verified at zero.
- Surfaces and equipment in the area are decontaminated with an appropriate disinfectant.
- All disposable PPE is removed and placed into a biohazardous waste container.
- Hands are washed thoroughly with soap and water.

## 10.0 Emergency Procedures

- Refer to department-specific policies and procedures.
- Injuries/exposures are reported according to department specific policies.
- In the event of a serious injury or emergency, call 911 from a campus phone or Emory Police at 404-727-6111 from a cell phone
- In the event of a spill, refer to the "Just in Time" Guide, or call the Emory University Environmental Health & Safety Office, 404-727-5922.

## 11.0 References

- Biosafety in Microbiological and Biomedical Laboratories 5th Edition, 2009. (<https://www.cdc.gov/biosafety/publications/bmb15/index.htm>).
- International Society for the Advancement of Cytometry (ISAC) Biosafety Standards. (<http://isac-net.org/PDFS/d2/d2a43c32-5f89-44d7-adf6-c137d38f81b1.pdf>).
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- 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.
- Rutala WA, Weber DJ, HICPAC. Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008. 2011.

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***Table 1.0 – Roles and Responsibilities for Approval for Cell Sorter Use***

	<b>ROLE</b>	<b>RESPONSIBILITIES</b>
Step 1	The Principal Investigator:	<ul style="list-style-type: none"> <li>• Completes a biosafety protocol (NOI).</li> <li>• Indicates on the protocol:               <ul style="list-style-type: none"> <li>○ Intention to cell sort.</li> <li>○ The agent.</li> <li>○ Which sorting facility will be used.</li> </ul> </li> </ul>
Step 2	The Biosafety Office:	<ul style="list-style-type: none"> <li>• Reviews protocol and conducts a risk assessment for the agent:               <ul style="list-style-type: none"> <li>○ Determines the BSL</li> <li>○ Develops a BARS if there is none</li> <li>○ Adds to the agent to Table 2.0.</li> <li>○ Sends the updated Table 2.0 to the sort operators.</li> <li>○ Updates the SOP.</li> <li>○ Provides any necessary training.</li> <li>○ Notifies Occupational health with any concerns.</li> </ul> </li> </ul>
Step 3	The Cell Sorting Operator Ensures:	<ul style="list-style-type: none"> <li>• Sorting activities have been approved by the Biosafety Office.</li> <li>• Involved personnel have completed appropriate training.</li> <li>• Necessary PPE is available.</li> <li>• Questions and concerns are directed to the Biosafety Office.</li> </ul>

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**Table 2.0 – Biosafety Level Determination for Cell Sorting**

CONTROLS	BSL2	BSL2+ (DURING SORTING OPERATIONS)	BSL3
Risk Assessment Condition	Uninfected non-primate	Non-infectious human/NHP cells	Infectious samples with high risk assessment
		Infectious but with low risk assessment	All samples containing known aerosol pathogens
Example Sample Type or Agents	<ul style="list-style-type: none"> <li>Normal murine cells</li> <li>3rd gen Lentivirus (non-human cells)</li> </ul>	<ul style="list-style-type: none"> <li>Normal human or NHP blood</li> <li>Human or NHP cell lines e.g., Influenza A</li> </ul>	Example agents include: <ul style="list-style-type: none"> <li>Mycobacterium</li> <li>Tuberculosis</li> <li>Monkey pox</li> </ul>
Aerosol Management System Validated	Periodically (monthly or with filter change)	Periodically (monthly or with filter change)	Before every Sort
AMS System	Required	Required	Required
N-95 Respirator	Optional	Required	Required
PAPR	Optional	Optional	Optional (as a replacement for N-95)
Eye Protection	Face shield or safety goggles	Face shield or safety goggles	Face shield or safety goggles (not needed with PAPR)
Lab Coat	Front Closure lab coat	Front Closure lab coat, wrap-around rear closure	Fluid-resistant coveralls
Separate Room and Environmental Controls	Optional	Required or limited access to room	Required

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**Table 3.0 – Biosafety Level Determinations**

***This list represents biosafety level determination for cell sorting of specific agents. However, final determination of the biosafety level is dependent upon the risk assessment conducted in collaboration with the Biosafety office and other subject matter experts.***

AGENT BEING SORTED	RECOMMENDED BIOSAFETY LEVEL	RESTRICTIONS OR COMMENTS	SDS LINK <sup>2</sup>
Hepatitis C	BSL2+		<a href="#">Hepatitis C</a>
Influenza A	BSL2+	Influenza (seasonal) vaccine recommended	<a href="#">Influenza A</a>
SIV, SHIV	BSL2+		
LCMV	BSL2+ or BSL3	Ensure that HVAC system does not exhaust near vivarium housing mice; BSL dependent upon strain; pregnant women should consult Occupational Health Services or their personal physician prior to performing a procedure with this agent.	<a href="#">LCMV</a>
Malaria	BSL2+ <sup>1</sup>		
Listeria	BSL2+	Pregnant women should consult Occupational Health Services or their personal physician prior to performing a procedure with this agent.	<a href="#">Listeria</a>
Vaccinia	BSL2+	vaccine recommended	<a href="#">Vaccinia</a>
H1N1	BSL3	H1N1 vaccine recommended	
HIV	BSL2+ or BSL3		<a href="#">HIV</a>
TB, Mycobacterium tuberculosis	BSL3		<a href="#">TB</a>

<sup>1</sup>Respirator optional (mucous membrane protection required) for this agent except where the sample also contains human/NHP blood cells or fluids

<sup>2</sup>EHSO Online MSDS Link ([EHSO SDS Online](#))