Guidance for Sorting and Analyzing Unfixed Biological Materials Using Flow Cytometry

Important Notice

COVID-19 screening of donors is required for sorting of unfixed, primary human materials collected prior to January 2020. Please contact the Biological Safety Office at Biosafety@augusta.edu for more information.

Purpose

The purpose of this document is to provide guidance to flow cytometry core directors/managers and laboratories utilizing flow cytometers on obtaining Biosafety or Institutional Biosafety Committee (IBC) approval to sort or analyze **unfixed** biological materials. The Biosafety Office and the IBC have collaborated on this guidance to ensure safety of the research community engaged in these operations and to facilitate the approval process. Please refer questions on this guidance to the Biosafety Officer at (706) 721-7458, or lmeyer@augusta.edu.

References

- 2014 International Society for Advancement of Cytometry (ISAC) Biosafety Standards.
- CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 5th Ed. Standard. (BMBL)

I. Requirements for Flow Cytometers with Stream-in-Air Sorting Capability (Sorters)

A. *Facility:*

i. Cores and laboratories wanting to sort unfixed biological materials will need to upgrade from Biosafety Level (BSL) 1 containment to BSL2. The ISAC Standard indicates that sorting unfixed uninfected non-primate cells (i.e. mouse cells) requires BSL2 containment. The requirements for this upgrade can be found in the BMBL (see reference above). In brief, the standard indicates the following BSL2 facility requirements:

- ✓ Laboratory door that closes and locks
- ✓ Sink for hand washing
- ✓ No porous furniture (i.e. cloth chairs)
- ✓ An eyewash station available within 10 seconds of equipment
- ✓ Negative pressure relative to the corridor/public areas
- ✓ Placards placed on the door indicating bio-hazardous materials in use (provided by the Biosafety Office).

ii. Cores or laboratories wanting to sort unfixed non-infectious human/non-human primate cell lines (including those from ATCC), primary human/non-human primate materials (i.e. blood) or other infectious materials with a low risk assessment (determined by the Biosafety Office) will upgrade to BSL2+. BSL2+ is Biosafety Level

2 requirements with enhanced precautions (BSL3 practices) during sort operations. BSL2+ requirements are listed in Section II.

iii. Augusta University is not equipped at this time to sort infectious samples with a high risk assessment (determined by the Biosafety Office) or samples containing known aerosol pathogens (i.e. Mycobacterium Tuberculosis). These experiments require BSL3 facilities and practices.

B. *Sorter Requirements:* Equip sorters with an Aerosol Management System (AMS). Test the AMS periodically or with every filter change. Develop a log to document testing. Include the testing procedure in the SOPs. The sorter operator will demonstrate the testing procedure to the Biosafety Officer.

C. *Standard Operating Procedure (SOP):* Each core or laboratory will develop an SOP for sorting. The SOPs will be submitted to the Biosafety Office for Biosafety or IBC review and approval. The core director/manager or Principal Investigator (PI) will periodically review the SOP. Updates will be submitted to the Biosafety Office for Biosafety or IBC review and approval.

D. *Intake Form:* Each core (does not apply to individual laboratories) will create a simple form that users will submit. The purpose of this form is to document the hazardous materials being brought into the facility, to ensure that the PI has Biosafety/IBC approval and to rule out some safety concerns (i.e. if the materials have been fixed or tested for any pathogens). For sorting the form can cover multiple experiments and users as long as the experiments involve the same sample types and no changes in hazards/risks. A new form should be completed for each sample type. To obtain Biosafety/IBC approval, PIs will submit an amendment to their Biosafety Protocol, using the amendment form available on the Biosafety or IBC website.

E. *Training:* The sorter operator will complete Initial Biosafety Training (required once) and the Annual Biosafety Refresher (required annually). The Biosafety Office can verify completion of this training.

F. *Personal Protective Equipment (PPE):* Sorter operators will wear gloves, button front lab coat and safety glasses.

II. Additional Sorting Requirements for BSL2+ Containment

A. *Facility:* Facilities used for sorting unfixed human/non-human primate or other infectious materials must meet one of the following criteria for:
   i. Place the sorter in a containment enclosure that ensures personal protection (i.e. negatively pressure, HEPA filtered), preferably a Biosafety Cabinet; **or**
   ii. Place the sorter in a separate room with limited access; **or**
   iii. If the sorter is located in a facility where other laboratory operations occur, or there are analyzers/other equipment present, access to the room shall be restricted only to those persons necessary to perform the sorting experiment, during sorting

operations. If other experiments must be performed, all persons present will adhere to all PPE, training and occupational health requirements outlined in this guidance document or access shall not be permitted.

B. *Signage:* Place a "do not enter – sorting in progress" or similar sign on the door leading into the facility when a sort is in progress.

C. *Training:* The sorter operator will complete Bloodborne Pathogen Training (required annually). The Biosafety Office can verify this training. Additionally, sorter operators require a minimum of 200 hours of experience sorting non-infectious (i.e. mouse cells) on the actual sorter that they will use. Operators cannot obtain the required experience using beads. The operator will need to develop a log to document experience hours (inclusive of the date/time, type of material sorted, number of hours for that experiment and issues that occurred). The log will be submitted to the Biosafety Office.

D. *Occupational Health*: Visit Employee Health and Wellness (706-721-3418) for a health screening (inclusive of TB testing) and Hepatitis B Vaccination series or to complete the vaccination declination form. Medical clearance and fit tested from Employee Health and Wellness must be obtained to wear a respirator. Depending on the hazards being brought into the facility the Biosafety Office or IBC may recommend additional vaccinations.

E. *PPE:* Sorter operators will wear gloves, a solid front lab coat (wrap around), goggles or a face shield and an N-95 or higher respirator. If the sorter is in a containment enclosure, the goggles, face shield and respirator are only required during instrument/sample manipulation outside of the enclosure. During sorting, this PPE can be removed if the enclosure and AMS are operational.

III. Requirements for Flow Cytometers without Sorting Capability (Analyzers)

A. *Facility:*

i. BSL1 containment is sufficient for analysis only of unfixed, uninfected non-primate cells (i.e. mouse cells).

ii. Cores or laboratories wanting to analyze unfixed non-infectious human/non-human primate cell lines (including those from ATCC), primary human/non-human primate materials (i.e. blood) or other infectious materials with a low risk assessment (determined by the Biosafety Office) will need to upgrade to BSL2. See facility requirements in Section I.A.i.

iii. If the analysis of cells utilizes a stream-in-air cytometer, the sorting requirements indicated above in Sections I and II must be followed based on the agents and BSL required to safely contain them.

iv. Augusta University is not equipped at this time to analyze infectious samples with a high risk assessment (determined by the Biosafety Office) or samples containing

known aerosol pathogens (i.e. Mycobacterium Tuberculosis). These experiments require BSL3 facilities and practices.

B. *Analyzer Requirements:* For work involving human/non-human primate materials and other infectious agents, the waste containers will be prefilled with concentrated bleach to provide a final 10% solution upon filling and emptied on a regular basis. Waste containers will be equipped with a hydrophobic 0.22um filter at the air vent. The system will be flushed with disinfectant after each use or periodically – as indicated by the Rules of Use.

C. *Intake Form:* Each core (does not apply to individual laboratories) will create a simple form that users will submit. The purpose of this form is to document the hazardous materials being brought into the facility, to ensure that the PI has Biosafety/IBC approval and to rule out some safety concerns (i.e. if the materials have been fixed or tested for any pathogens). For analysis the form can cover multiple experiments and users as long as the experiments involve the same sample types and no changes in hazards/risks. A new form should be completed for each sample type. To obtain Biosafety/IBC approval, Principal Investigators will submit an amendment to their Biosafety Protocol, using the amendment form available on the Biosafety or IBC website.

D. *Rules of Use:* Each core or laboratory will develop "Rules of Use" for analyzers. The Rules of Use will be submitted to the Biosafety Office for Biosafety or IBC review and approval. The core director/manager or PI will periodically review the Rules of Use. Updates will be submitted to the Biosafety Office for Biosafety or IBC review and approval. Each user will signify understanding by signing the signature page of the Rules of Use document.

E. *Training:* All users will complete Initial Biosafety Training (required once) and the Annual Biosafety Refresher (required annually). Users who want to analyze human/non-human primate materials will need to complete Bloodborne Pathogen training (required annually). The Biosafety Office can verify completion of this training.

F. *Occupational Health:* For work involving human/non-human primate materials, visit Employee Health and Wellness (706-721-3418) for a health screening (inclusive of TB testing) and Hepatitis B Vaccination series or to complete the vaccination declination form.

G. *PPE:* Users will wear gloves, button front lab coat and safety glasses.