

INTRODUCTION

The University of Wisconsin Carbone Cancer Center (UWCCC) Flow Cytometry Core Facility supports basic, translational and clinical research as well as provides instrumentation assistance and experimental design consultation services for cell sorting and analysis for campus and off-campus researchers.

The core facility is a shared service BSL-2 facility with two sorters: a BD FACSAria II cell sorter with an aerosol management system and a BD FACSAria III with a Baker BioProtect biosafety cabinet. The samples processed at the facility may contain both known and unknown pathogens. Since many sorted samples are unfixed and because sorters generate aerosols, risk assessment and communication are important to ensure the safety of facility staff and users.

The UWCCC Flow Cytometry Core Facility contacted the Office of Biological Safety (OBS) to request guidance and support for the facility. To enhance communication between the facility and researchers, in addition to providing recommendations for containment and personal protective equipment, OBS developed a risk assessment document. The document is flexible so that it can be used by the facility as well as individual laboratories owning flow cytometry equipment.

METHODS

While creating a UW-Madison specific flow cytometry risk assessment document, several regulatory and guidance documents were consulted including the following:

- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)
- Biosafety in Microbiological and Biomedical Laboratories (BMBL)
- International Society for Analytical Cytology Biosafety Standard
- UW-Madison IBC Handbook
- UW-Madison Biohazard Recognition and Control: Guidelines for handling pathogenic microorganisms and disposing biohazardous waste

We also consulted flow cytometry experts and other universities to learn about the practices and procedures in place at their flow cytometry facilities.

A thorough site visit was conducted to inspect the UWCCC Flow Cytometry Core Facility and to better understand the work flow. We participated in user trainings, reviewed the core's SOPs, and reviewed the facility's umbrella biosafety protocol.

Sample Type	Characterized Human Cells *2 ❖ RG2 organisms not known to infect via aerosol ❖ Opportunistic Pathogens (IBC defined)*3 ❖ Some cells transduced with viral vectors *4	Human cells *2 ❖ Some cells transduced with viral vectors*4 ❖ RG2 organisms infectious via aerosol *5 ❖ Transfection method unknown ❖ Proprietary cells/samples	Human cells *2 ❖ Some cells transduced with viral vectors*4 ❖ RG2 organisms infectious via aerosol *5 ❖ Transfection method unknown ❖ Proprietary cells/samples
❖ Non-Infectious mammalian cells ❖ Fixed human cells ❖ Fixed NHP cells ❖ Plant cells*1 ❖ RG1 organisms	BSL-1 *6	BSL-2 *6	BSL-2 *6
None required	Aerosol Management System or BSC	BSC	Aerosol Management System (with additional precautions)

RESULTS

Because of aerosol generation during cell sorting procedures, there are special considerations when determining the risk associated with the experiment. A risk assessment document was created that can be applied to flow cytometry facilities on the UW-Madison campus. In addition to sample, equipment, and PPE assessment, the document provides information regarding flow facility pre-approval, transport of biological material, waste disposal, equipment disinfection, spill procedures and reporting requirements. The guidance document is applicable to BSL-1 facilities and BSL-2 facilities that meet the additional facility requirements outlined in the ISAC Biosafety Standard for Sorting of Unfixed Cells.

When performing a risk assessment for cell sorting activities, many variables should be considered, including but not limited to: the sample type, sample preparation, the biosafety level of the facility, available equipment for aerosol containment, experience/training of personnel, and PPE. The risk assessment document provides a framework to consider these factors, however it is not definitive. A risk assessment should be performed by the research team with consultation from both the flow cytometry facility and the Office of Biological Safety. The IBC determines the minimum requirements needed for each type of sample.

CONCLUSIONS

- Improved communication between
 - Flow lab personnel and OBS
 - OBS and researchers
 - Researchers and flow lab personnel
- Increased acceptance of samples types, expanding the core's client base
- Change in practices based upon risk assessment
 - Increased safety precautions for subcategories of samples to better protect personnel
 - Adjusted safety precautions when containment equipment used
- Increased awareness of sample content allowing the core to assist researchers to create better experimental designs and results
- Improved consistency between work listed in biosafety protocols and research being performed

ADDITIONAL INFORMATION

Office of Biological Safety, UW-Madison:
<http://www.ehs.wisc.edu/biosafety.htm>

1. Plant cells of which the parent plant was not infected with microbes or substances requiring a permit of any kind. Other plant cell sorts should be assessed for risk on a case by case basis.

2. Screening: Contact FACS facility to inquire if any specimen screening is required.

3. UW-Madison, Office of Biological Safety (OBS) – Institutional Biosafety committee (IBC) Handbook, IBC Policy on Appropriate Containment for Opportunistic and Borderline Pathogens, located at the OBS website www.biosafety.wisc.edu

4. Viral Vectors: Risk assessment should be detailed to include analysis of the viral vector system used for transfection of the cells.

5. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH) – Biosafety in Microbiological and Biomedical Laboratories (BMBL), current version and/or any subsequent revisions <http://www.cdc.gov/biosafety/publications/bmb15/>.

6. A higher biosafety level may be required if the space is shared: UW-Madison, Office of Biological Safety (OBS) – Institutional Biosafety committee (IBC) Handbook, IBC Policy on Shared Lab Spaces, IBC-POI-008, located at the OBS website www.biosafety.wisc.edu.