



Flow Cytometry Shared Resource

INTRODUCTION

This document is for the attention of all Principal Investigators and Flow Cytometry Shared Resource (FCSR) users. The number of our users and the diversity of their applications continues to expand, as demand grows and the technology advances. Here we will review and update, on an annual basis, the guidelines which are in place for the use of this facility. Please read these guidelines, become familiar with them, and share them with your staff.

The FCSR is an open facility, available to internal university users, as well as external academic and commercial institutions. The FCSR is open access, between normal business hours of 9:00am – 5:00pm (Eastern Time) Monday through Friday, with 24/7 access available to approved trained users (internal university users only).

The FCSR has the authority to establish these policies and has control of the associated Standard Operating Procedure (SOP) documents. The FCSR is responsible for the implementation of these policies, as well as for ensuring that facility staff and users understand, acknowledge and comply with these policies. Target users are FCSR staff, internal (university members) and external (academic and commercial) users of the FCSR.

Use of the Flow Cytometry Shared Resource is for **RESEARCH PURPOSES ONLY**.

FACILITY AND INSTRUMENT ACCESS

Prerequisites

All users are required to register in the iLab management system and to have deposited the mandatory Billing Authorization document (see below) with the facility each month, prior to obtaining online scheduling privileges through iLab and subsequent instrument access. We recommend that all instrument operators attend the two day *Basic Flow Cytometry Course*, prior to being granted instrument access. This course is offered, free of charge, on the second Tuesday

and Wednesday of each month, by the FCSR. Users are encouraged to complete the course; however, it is not mandatory for access to the facility within normal business hours. The training course is a mandatory requirement for those users seeking after-hours access. Users must then demonstrate to facility staff that they understand and know all of the appropriate procedures in order to use each instrument. These include turning the instrument on and off, handling the necessary fluidics systems, and correctly running samples. Users with after-hours access take full responsibility for the instrument(s), and any repairs needed after misuse of an instrument will be billed to their Principal Investigator. Trained and vetted internal university members may access the facility 24 hours a day, 7 days a week. This access is limited to the cell analyzers only. External users will not be granted after-hours access. Independent use of the facility requires that users be proficient in instrument operation and basic maintenance, including filling the sheath and emptying the waste reservoirs. The FCSR staff has final authorization and approval of individuals wanting to use the facility outside of normal working hours.

Billing authorization form

A new “Billing Authorization Form” is due at the beginning of each month, in order for users to have access to the facility. This form gives us authorization to bill the account(s) listed, for services rendered within the designated calendar month, and must be submitted before the facility is able to render any services. Failure to submit a “Billing Authorization Form” will result in blocked access to the iLab instrument reservation system. Access will be restored once a form has been submitted.

MAINTENANCE AND CARE

The FCSR staff perform daily Quality Control, via Cytometer Setup and Tracking (CST) beads, for all instruments within the facility, and perform daily aseptic cleaning of the Cell Sorters, in preparation for sorting. Bacterial contamination testing is performed weekly to confirm sterility of the instruments, and monthly decontaminations ensure a deep clean of the entire fluidics systems. Users are expected to be familiar with and be able to perform correctly: startup, shutdown, and cleaning procedures on all the instruments which they plan to use. This includes cleaning up the work surfaces, discarding waste, refilling sheath fluid reservoirs and emptying waste containers, in addition to cleaning the sample injection port with FACS Clean and water following the completion of sample acquisition. Users should report all instrument problems to a FCSR staff member, especially, if such issues arise during after-hours use.

SAFETY AND BIOSAFETY

Regulations and Guidelines

All cell analyzers and the FACS Aria-II (BSL-1) cell sorter operate under Biosafety Level 1 (BSL-1) conditions ONLY. If Biosafety Level 2 (BSL-2) samples need to be acquired on the cell analyzers, they must be fixed and rendered non-infectious using an appropriate fixative (4% paraformaldehyde, 4% formaldehyde, or 10% formalin; alcohols are not adequate in rendering

BSL-2 cells to BSL-1). The FACS Aria-IIu (BSL-2) and FACS Aria Fusion (BSL-2) cell sorters can process Biosafety Level 2 cells. Samples which require BSL-2 containment include all “normal” live human samples, human patient samples and cells transfected with retroviral/lentiviral vectors capable of infecting human cells. Biosafety Level 3 and Biosafety Level 4 cells **CANNOT** be processed on any instrument within the facility. Clinical samples **CANNOT** be processed in the FCSR. However, clinical samples designated “FOR RESEARCH ONLY” are acceptable and must be fixed, if using the analyzers, or processed on the BSL-2 sorter.

Biosafety Questionnaire

All laboratories must fill out a Cell Analysis Questionnaire, regarding the types of samples that will be analyzed or sorted in the facility. This is performed through iLab and must be completed annually, no later than the end of January each year. Access to the facility may be delayed or denied if a completed questionnaire is not on file. Any changes in the types of experiments, cell types, and other information pertinent to the safe use of the facility must be discussed with and communicated in writing to the FCSR prior to undertaking these new studies. Supporting documentation must also be provided, where necessary.

Instrumentation

Use of the FCSR instrumentation is restricted by Biosafety Level (BSL). All of the analytical flow cytometers – FACS Canto-II, LSR-II, and LSR-Fortessa-HTS, as well as the FACS Aria-II cell sorter, are restricted to samples that are handled under Biosafety Level I (BSL-1) conditions only. The FCSR has a Baker BIOPROtect II cabinet, housing the FACS Aria-IIu cell sorter, which may analyze or sort samples under BSL-2 conditions.

Samples

Samples which require handling under BSL-3 or BSL-4 conditions are never permitted in the FCSR.

BSL-2 samples that have been fixed are allowed to be analyzed on the BSL-1 flow cytometers. It is the responsibility of the Principal Investigator to ensure that students, employees and trainees, are properly instructed in appropriate fixation protocols and that all personnel carry out these protocols correctly.

Users who plan to analyze human cells (live, or fixed), **MUST** designate that fact when reserving time on the pertinent flow cytometer in the iLab system. Similarly, genetically modified (virally transduced) cells should also be identified. This signals to subsequent users and FCSR staff that these type of samples will be processed. Appropriate instrument decontamination procedures must also be followed after any such analysis.

Safety

All users are instructed to apply Universal Safety Precautions and wear appropriate Personal Protective Equipment, when handling samples in the FCSR.

All users are expected to take the *Blood-Borne Pathogens Course*, administered through the Office of Environmental Health and Safety. Fulfillment of this requirement by users is the responsibility of their Principal Investigator.

No “Sharps” are permitted in the FCSR.

INSTRUMENT ACCESS

Prerequisites

All users are required to be registered in the iLab management system, prior to obtaining online scheduling privileges and instrument access, as well as to have deposited the mandatory Billing Authorization document with the facility at the start of each month.

It is required that all instrument operators attend the two day *Basic Flow Cytometry Course* prior to being granted instrument access, unless they are able to demonstrate appropriate previous experience, obtained elsewhere. The FCSR course is offered, free of charge, on the second Tuesday and Wednesday of each month. Independent use of the facility requires that the user be proficient in instrument operation and basic maintenance, including filling sheath and emptying waste reservoirs. The FCSR staff has final authorization and approval of individuals wanting to use the facility outside normal working hours.

Instrument reservations and cancellations

Instrument reservations policy

Reservations must be made in iLab, through the user’s iLab account, or by using the iLab Kiosk to make a walk-up reservation. Users should **NOT** make reservations for other members of their lab, or for anyone else. Users accept full responsibility for the instrument during their scheduled reservations and the reservation owner will be held accountable for any misuse of the instrument. Repairs needed because of instrument misuse will be billed to their Principal Investigator. Unregistered users are not permitted to operate the instruments, therefore, reservation owners must be present during the entire sample acquisition period. Reservations for the analyzers can be made up to 30 days in advance and walk-up reservations can be made on the spot, using the iLab Kiosk. Reservations for the cell sorters are subject to approval and may be made up to 21 days in advance. Instrument reservations are billed for the scheduled time, or the actual time used, whichever is the greater. The facility will cancel a reservation if the user/lab has not completed or submitted all the necessary documents that are required for continued access.

Missed reservations and late arrivals

Missed reservations are billed for the entire scheduled time. Users will be billed for the portion of unused reserved time, after late arrival. Extended time beyond their original scheduled reservations will not be granted, unless there is time available on the instrument.

Cancellation policy

For the analyzers, users may cancel or edit their reservation, up to 30 minutes prior to their reservation start time. Cancellations are not allowed within the 30 minutes prior to a reservation start time, and the reservation will be billed for the full scheduled time. **EXCEPTION:** reservations longer than 30 minutes will be adjusted and billed for the first 30 minutes; the remainder of the reservation time will become available to other users on the instrument calendar. For the sorters, reservations must be cancelled 24hrs in advance (at least the day before) and users must contact the facility staff to cancel their reservation. Cancellations cannot be made on the day of the scheduled sort and the reservation will be billed for the entire scheduled time. **EXCEPTION:** consideration will be given to extreme circumstances that could lead to a last minute cancellation and billing of either the full or a fraction of the reservation may be waived.

INSTRUMENT MAINTENANCE

Routine maintenance of the FCSR instrumentation is critical for optimal data collection. The FCSR performs daily Quality Control, via Cytometer Setup and Tracking (CST) beads and routine maintenance on all instruments. It is not only important, but the responsibility of the FCSR users, to become familiar with and be able to perform correctly: startup, shutdown, and cleaning procedures on the instruments that they will be using, as well as refilling the sheath fluid and emptying the waste container.

FCSR users are expected to clean-up the work area which they have used, upon completion of their analysis, including refilling the instrument's sheath fluid reservoirs and emptying the waste containers, as necessary. **IMPORTANT:** bleach is to be added to emptied waste containers (>10% final volume), before reconnecting the container to the instrument. FACS Clean solution and then water are to be run through the flow cytometer, following completion of analysis, for two minutes each – **NO EXCEPTIONS!**

Spills or drips of any kind must be cleaned and disinfected immediately after occurrence. A disinfection/clean-up kit is located in the FCSR. Equipment, tips, sample tubes, tube racks, papers, etc. require removal upon completion of analysis. Items left behind at the end of the day will be discarded. All bio-hazardous waste is to be placed in containers with red biohazard waste bags.

INSTRUMENT ISSUES AND PROBLEMS

Always report any instrument faults, errors, problems, or issues, no matter how small or seemingly insignificant, to a FCSR staff member immediately. This is especially important if something occurs after hours, when a staff member is not immediately available by telephone. Users are expected to understand the meaning of instrument warning lights, alarms, etc. and to act accordingly when these may be activated.

DATA MANAGEMENT

Policy

The UM SCCC Flow Cytometry Shared Resource is not responsible for managing and storing acquisition data files. It is the user's responsibility for backing up and managing permanent storage solution of data. Instrument workstations are purged frequently, in order to ensure reliable and efficient instrument operation; therefore, they are not considered to be a permanent storage method. Data management and storage is the personal responsibility of each respective user.

Data storage options

Facility data server, SCCC FCSR (S:).

University cloud-based platform, BOX (box.miami.edu).

Other cloud based storage solutions (ex. Google Drive, etc...).

NOT ALLOWED are removable drives (e.g. flash drive or external hard drives) due to the potential virus contamination risk through the USB port.

CITATIONS AND PUBLICATIONS

Acknowledgments

Principal Investigators/Laboratories agree to acknowledge the UM SCCC Flow Cytometry Shared Resource in all publications and grant applications, where data generated by use of the facility is presented. This includes abstract and journal publications, poster presentations and seminar speeches.

Acknowledgment example: "We would like to acknowledge the skilled assistance of the Flow Cytometry Shared Resource of the Sylvester Comprehensive Cancer Center at the University of Miami, Miller School of Medicine, for the provision of expert fluorescence analysis and cell sorting services".

Co-Authorship

Publication co-authorship with a Flow Cytometry Shared Resource staff member should be considered, where there was a significant contribution to the experimental design, or final outcome of the study.

Notification

The FCSR strives to maintain a library of publications that have been made using our services. Please always send the FCSR an electronic copy of your newly accepted papers, if they contain data generated in the FCSR. Please also let the facility know when any grants, which contain FCSR-generated data are awarded.