

Standard Operating Procedures (v6 6/11/19)

Safe Working Practices for Flow Cytometry on the FACSCalibur

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I. General Facility Information

The OUHSC Image and Flow Cytometry Laboratory is located on the 3rd floor Room 1317 of the Biomedical Research Center with the main office located on the 1st floor, Room 1106. Regular business hours are between 9:00 AM to 5:00 PM Monday through Friday, but after-hours card access to facilities may be granted for experienced users upon request. Facility doors are locked from 5:00 PM through 8:00 AM. The laboratory is approved at Biosafety Level 2 (BSL-2) with restrictions regarding anything higher than BSL-2. Staining procedures, which do not require washing steps (i.e. live/dead stain) may be completed at the flow facility in the biosafety cabinet. The laboratory is maintained under negative pressure at all times. Laboratory door is always closed, and gloves and lab coats must be worn when handling samples and operating the FACSCalibur.

II. Facility Orientation and Training

Facility staff provides training and orientation for all instrumentation. New users are required to take a one-on-one, 2 hour, hands-on training on the relevant instruments. Exposure procedures and emergency response are discussed with our users during training. Exemption from hands-on instrument training is given to users who are only signing up for assisted appointments, but lab personnel will still go over safety guidelines. Additionally, we will provide each user with a copy of the SOP and will require a signed confirmation of receipt and content. Furthermore, copies of all SOPs are available in the laboratory and on the facility website so that they can be easily accessed at any time. Training on the FACSCalibur instrument includes basic instrument operation and analysis as well as all relevant safety procedures, spill management, and decontamination.

III. Startup Procedures

1. Check fluid levels for sheath tank and waste tank (with 10% bleach). **Waste levels must be manually monitored –do not allow waste container to overflow!** Empty if tank is $\frac{3}{4}$ full.

2. Turn on the FACSCalibur (green button located on right side of instrument).
3. Restart the computer if you are the first user for the day.
4. Start Cell Quest program and load instrument settings as well as the acquisition document as described for manual loading. Note: Staff will assist users in building new experimental method files during assisted appointments only.
5. If mixing of samples is required, all tubes **must be capped or covered with paraffin before vortexing to minimize aerosols (placing glove over tube is not acceptable).**
6. If using the automated carousel workstation be sure that the plexi-glass shield with magnet is placed correctly on the base of the unit and fingers and loose objects are not touching the carousel during operation. **Users are to report any suspected damage to the instrument prior to running samples. If there is any damage found after usage, you may be held accountable!**

IV. Shutdown Procedures

1. Export your data to your storage device from the Calibur Data folder.
2. Check the web calendar to see if there is anyone using the instrument for the day. **If you are the last user you are required to correctly shutdown the FACSCalibur!**
3. If you are not the last user just leave the machine turned on and close your experiment.
4. For shutdown you will need to run 5 minutes of 10% bleach and 5 minutes of DI water.
5. Discard all tubes into the biohazard container and wipe down counter top with 70% ETOH or ConFLIKT.
6. All user and staff must wash hands and remove lab coats before leaving the laboratory.

V. Spill Procedures

Flow cytometer surfaces are wiped with 10% bleach, left on surface for 5 minutes followed by a water rinse with deionized water. Large spills on flow cytometer surfaces or on other lab surfaces are cleaned with 10% bleach left on surface for 20 minutes with a soaked, wet towel. We require all users to follow guidelines in our spill protocol when working at our facility. Spill kits are provided in the laboratory. 10% bleach solution (made daily) is provided on all bench-tops. PPE (gloves) and other items used for clean up should be properly disposed of in biohazard containers after cleaning of a spill.

VI. Exposure to Biohazardous Material

All employees with occupational exposure to human blood, tissues, or cell lines are required to take the online EHSO bloodborne pathogen training course at the time of assignment to tasks where occupational exposure may take place, when changes affect employees' occupational exposure, and at least annually thereafter. The hepatitis B vaccine should be made available to all employees who have occupational exposure to blood or other potentially infectious materials. If an employee sustains an exposure

incident (such as a stick with a contaminated needle/scalpel/dental wire or a splash of potentially infectious material in the eye, mouth, mucous membrane, or non-intact skin), the exposed person should immediately:

- a. Clean the wound with soap; flush mucous membranes with water or normal saline solution.
- b. Notify his/her supervisor, designated coordinator, or other designated individual.
- c. Proceed for treatment within 1-2 hours of the exposure (see the OUHSC/OU-Tulsa Infectious Diseases Policy for current recommended treatment locations).
- d. If possible, for laboratory exposures, bring a sample of the source material to the treatment facility for testing.

Sources: OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030)

The following facilities are recommended for treatment of occupational injuries or exposures, however employees may choose any health care professional they wish.

Employee Health

OU Physicians Building Suite 2C

825 NE 10th

Oklahoma City, OK

271-9675 (271-WORK)

Hours: 8:00 a.m. – 4:30 p.m. Monday through Friday (Call before going to the clinic)

OU Medical Center Presbyterian Tower Emergency Room

700 NE 13th Street

Oklahoma City, OK

405/271-3667

Hours 4:30 p.m. - 8:00 a.m. Monday - Friday and weekends

Other useful links:

Institutional Biosafety Committee (IBC) (<http://www.ouhsc.edu/ibc/default.asp>)

EHSO General Employee Guidelines (<http://www.ouhsc.edu/ehso/employee12.html>)