



Flow Cytometry Core Facility

Safe Operating Procedures for the MoFlo XDP

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I. Purpose

The purpose of this safe operating procedure is to define the methods of proper and safe usage of the equipment in the Flow Cytometry Core Facility. The procedures listed are meant to supplement OMRF's Chemical Hygiene Plan and the Biosafety Manual. All procedures listed are meant to ensure the safety of all users, allow correct usage of equipment and to prevent cross contamination. SOPs will be available in the facility and online.

II. General Facility Information

The Flow Cytometry Core Facility is located on the 4th floor in the Chapman Building in room E402. Regular business hours are between 8:30 am to 5:00 pm, Monday thru Friday. After hour usage is permitted for trained users whose badge has been activated with the badge scanner. The door must remain shut at all times. Signage listing the Bio Safety Level, emergency contact and Laser Radiation Warning must remain at the main entrance. The laboratory is approved at Biosafety Level 2 with restriction regarding live cell sorting on specific BLS-2 samples, which are classified as BLS-2+ or higher when in aerosol and other sources that contain viruses or other agents that are infectious to humans. Staining procedures, which do not require washing steps, may be completed in the Clean Bench hood. Gloves are required for operating the MoFlo XDP. Lab Coats are required for users with BSL-2 samples. PPE (gloves) will be available for all users. PPE (N95 respirators and eye protection) will be worn with nonfixed human cells or cell lines. No food or beverage will be allowed in the laboratory. All gloves will be removed and discarded before leaving the facility.

III. Facility Orientation and Training

Only facility staff will operate the MoFlo XDP. Staff will discuss the safety guidelines, exposure procedures and emergency response with all new users to the facility. All SOPs will be available in the laboratory and also available on line.

IV. Approval to Sort

Sorting appointments must be confirmed by staff on an individual basis. On request forms, Users/PI's must disclose any relevant information regarding individual samples prior to delivering samples for sorting. No live samples beyond BLS-2 can be brought to and processed in the facility.

New users are required to fill out and submit a "Pre-Sort Questionnaire – MoFlo XDP" no later than 24 hours prior to the appointment. This approval form must be resubmitted before new experiments or if the experiment protocol is altered in any way. Copies of these forms will be kept in the Flow Core.

V. Pre-Sort Procedures

Individual sorting requests are confirmed by the staff prior to appointments after ensuring that all required information and documentation has been provided and is up to date. Users will receive a confirmation email if approval is granted. Sort requests without proper information, IBC approval or with any conflicting condition will be denied. Users will receive an email about the denied request and can petition the denial with the facility staff. Only approved sort samples may be delivered to the facility for sorting. Samples must be completely processed, stained and ready to

acquire prior to sorting. Staining procedures, which do not require washing steps (i.e. live/dead staining) may be completed at the Flow Core in the Clean Bench hood. Samples must be filtered prior to sorting to reduce the possibility of clogging the instrument. Samples need to be covered when vortexing to minimize aerosols.

Flow Staff performs the following procedures prior to sorting:

1. Ensure the interlock on the collection chamber door is in place.
2. If using the Aerosol Management System (AMS), verify that the AMS is operating properly (suction control set at 20% and the time remaining must allow for the completion of the sort, otherwise the AMS filter and tubing needs to be replaced).
3. Pour 50mL of 10% Triton-X into waste tank. Ensure that the sheath tanks is appropriately filled.
4. Close sort collection chamber.
5. Ensure the proper nozzle is in place.
6. Start the flow cytometer (verify Nitrogen gas has adequate pressure and that house vacuum is on and all lasers turn on), computer and touch screen monitor.
7. Turn sheath on and debubble filter and purge sheath lines of air.
8. Stabilize stream and run daily quality controls.
9. Stabilize side streams, find phase and determine drop delay with flow check beads via puddle sort on glass slide. Place sort streams in appropriate position for sort.

Prior to aseptic sorts, surfaces of the sample injection chamber and the collection chamber are sterilized with 70% Ethanol. 10% bleach and sterile PBS are run through the flow cytometer prior to placing sterile sample on the sorter.

Prior to sorts for RNA preparations, surfaces of the sample injection chamber and the collection chamber are wiped with user provided RNase deactivating solution and user provided RNase deactivating solution (i.e. DEPC- treated water or RNase Away) is run through the flow cytometer prior to placing sample on sorter.

VI. Post-Sort Procedure

After sorting of any material, 10% bleach is run through the cytometer for a minimum of 5 minutes followed by a minimum of 5 minutes of distilled H₂O or sterile PBS. The sample station, collection chamber and the collection device are surface decontaminated with Conflit solution with a contact time of 5 minutes, computer bench is wiped off with 70% Ethanol (see inserted table).

Stream is shut off and appropriate fluidics shut-off procedures are followed. Instrument and computer are turned off. The waste tank (containing 50mL of 10% Triton-X) is emptied into the sink. The sink is rinsed with running water for 1 minute.

All users and staff are required to dispose of any PPE and wash and sanitize hands prior to leaving the laboratory.

Material to be Disinfected/Inactivated	Disposal method/Procedure (e.g., autoclave, disinfectant type, or picked up for off-site disposal by Safety Dept)	Disinfectant concentration and exposure time, or autoclave time and temp
Surfaces (counters and equipment)	Surfaces – 5 min 10% bleach or 5 min "Konflikt" Spray or 70% EtOH	10% Bleach or Konflikt spray or 70% EtOH for surfaces for 5 min, then wipe/rinse with water if bleach was used 70% EtOH for BSC interior for 5 min
Cell lines, infected material, rDNA materials	Left over blood disposed of in biohazard trash. Cell lines are decontaminated with bleach prior to disposal. Bleach is thoroughly rinsed from items before autoclaving.	20 minutes – 10% bleach 121°C (250°F), 15 psi for 60 minutes
Plasticware and glass	Blood contaminated pipette tips and consumable materials expelled into or soaked in bleach prior to disposal in biohazard trash. Bleach is thoroughly rinsed from items before autoclaving.	20 minutes – 10% bleach 121°C (250°F), 15 psi for 60 minutes
Animal bedding/wastes	NA	
Other: inside equipment	Sorter & Flow Cytometer Tanks	Sheath Fluid Tank – treated thoroughly with 70% EtOH for 20 minutes (tank closed), then rinsed with ddH ₂ O. Waste Tank - 10% bleach added to treat waste fluid during collection (or for at least for 20 minutes). Bleached waste fluid is diluted and discarded at sink. Flow cytometer & Sorter fluid lines are routinely rinsed with 10% bleach for 5 minutes for decontamination at before and at the end of daily operations and following the acquisition of any potentially hazardous live cells.

VII. Unexpected Stream Shutoff During Sort Procedure

Clogging or air bubbles may suddenly disrupt the stream and result with a deflection of sort or waste streams, spills into the collection chamber and significant vapor and aerosol generation. In order to restart the stream and resume sorting, the sort chamber will need to be opened in order to clean and dry all surfaces. The aerosols will need to be evacuated from the sort chamber (turn AMS to 100% for at least 2 minutes) and the sorting chamber needs to be decontaminated (clean the collection chamber surface with 10% bleach for 5 minutes, wipe off with towel and rinse with water).

Goggles and N95 respirators are strongly recommended for this procedure when BLS2 or samples associated with aerosol hazard are sorted.

VIII. Spill Procedures

Spill management is done following procedures outlined in the table below. Spills inside flow cytometers and on the flow cytometer bench are treated as Spills Outside Containment.

Flow cytometer surfaces are wiped with 10% bleach and left on surface for 5 minutes and then rinsed with water. Large spills on flow cytometer surfaces and inside sort chamber or on other lab surfaces are cleaned with 10% bleach and left on surface for 20 minutes with a soaked, wet towel and then rinsed with water. No spray bottles are allowed when cleaning a spill of any material associated with aerosol hazard. We require all users to follow guidelines in our spill protocol when working at our facility. 10% bleach solution is provided on all bench-tops. PPE is properly disposed after cleaning a spill.

Spills Outside Containment

Initial Response	<ul style="list-style-type: none"> • Evacuate if necessary • Alert co-workers and facility users and leave lab area immediately • Determine if medical attention is needed (injury, direct or potential exposure) • Call 911 for emergency response or HR at 271-7430 for non-emergency treatment • Close door and post lab with DO NOT ENTER sign • Remove and put contaminated garments into a container for autoclaving • Wash hands/face with soap or antimicrobial agent
Clean Up Response	<ul style="list-style-type: none"> • Wait at least 30 minutes before re-entry to allow aerosols to dissipate • Wear PPE upon re-entry (double gloves, lab coat, eye protection) • Cover spill with disinfectant soaked paper towels and pour an appropriate disinfectant solution around spill (10% bleach) and let stand for at least 20 minutes • Wipe up excess disinfectant and dispose appropriately • Use broom and dust pan to clean up any sharps and broken glass and contaminated materials when possible
Wrap Up	<ul style="list-style-type: none"> • Remove and discard PPE • Wash hands with soap or antimicrobial agent • Autoclave appropriate contaminated materials • File an Incident Report with Safety Department

IX. Waste Management

Empty flow cytometer waste tanks are filled with 50mL of Triton-X prior to starting instrument. Full waste tanks or during Post Sort Procedures the waste is emptied in to sink and the sink is rinsed for 1 minute. Unused samples of <1mL are capped and disposed in biohazard containers. Larger volumes should be treated with 10% bleach for 30 minutes prior to disposal in the sink which is then rinsed for 1 minute. Solid waste is disposed in biohazard bags.

X. Aerosol Management System

The Aerosol Management System is designed to reduce risk for exposure during cell sorting. A vacuum source creates negative pressure around the sort chamber and evacuates aerosols generated by the sort stream. The AMS Virosafe Filter is replaced when the time remaining status is at 00:00 or after 6 months. Performance is tracked by monitoring the airflow and by testing the escape of generated aerosols by the Glow-Germ assay. The Glow-Germ assay will be performed periodically to verify proper function of the AMS. The Glow-Germ assay is performed by covering the waste catch to deflect the waste stream. The instrument is set to 60 psi and the flow rate is set to achieve 20,000 particles/second. Measurements are taken using glass slides inside an Aero Tech air sampler in various positions around the sort chamber, collection chamber and at the exhaust of the AMS unit. Measurements will be taken at 5 minute time points to allow Glow-Germ particles to accumulate on the glass slides. Less than 1 particle per slide, under the condition of proper instrument function with AMS, will be considered a verification of properly working AMS. Records will be saved and kept with the instrument.

XI. Operator Training and Experience

All facility users are required to familiarize themselves with the policies within this SOP. Copies of this SOP will be available in the core facility and on-line. Only facility staff operate the MoFlo XDP.

XII. Exposure to Biohazardous Material (Emergency Guide)

In the event of an exposure to biohazardous materials, the following steps have to be taken: attend to the exposure or wound with first aid. If the injury is severe, call 911 or, if during working hours, call HR at 271-7430 and HR will coordinate non-emergency medical treatment. For injuries or exposures after hours, proceed to the nearest emergency room and contact HR the next working day. If a work related illness or injury occurs after hours, seek medical treatment at St. Anthony Hospital ER, 100 N Lee, Oklahoma City, OK 73101 (272-6152) or OU Medical Center, 700 NE 13th Street, Oklahoma City, OK 74104 (271-4064). The affected employee should also complete an Incident Report or Sharps Incident Report when able and submit it to the Safety Office.

Emergency Numbers

Safety Office	271-7266 600-8784 cell
Oklahoma Poison Control Center	1-800-522-4611