

Standard Operating Procedures (v9 5/1/17)

Safe Working Practices for Cell Sorting on the Influx

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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 8:00 AM to 5:00 PM Monday through Friday, but after-hours card access to facilities may be granted for trained 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 restriction regarding cell sorting on any unfixed cells derived from human or primates and unfixed cells from these and other sources that contain viruses or other agents that are infectious to humans. The laboratory is maintained under negative pressure at all times. Laboratory door is always closed and gloves are to be worn when handling samples or operating any instruments in the laboratory. Goggles or safety glasses, gloves, and lab coats are required for operating the Influx cell sorter and are made available for all users. Respirators (N95) are available, but not required, due to the cell sorting restrictions mentioned above.

II. Facility Orientation and Training

Facility staff provides training and orientation for all instrumentation. New users are required to take a one-on-one, hands-on training for the relevant instrumentation. 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 and will provide each user with a copy of this document. In addition, all SOPs will be made available on our laboratory website (<http://research.ouhsc.edu/core-facilities/flow-cytometry-and-imaging-2/standard-operating-procedures/>) so that they can be easily accessed at any time.

III. Approval to Sort

Sort appointments request must be confirmed by staff on a individual basis. On request forms, PI's

must grant access to their biosafety protocols and disclose any relevant information regarding individual samples prior to delivery of samples for cell sorting. All users are required to submit a "Cell analysis questionnaire for use of the Influx cell sorter in the flow cytometry and imaging core facility" prior to their appointments. This intake form must be submitted before experiments are initiated or experimental protocols are altered in any way. Copies of these intake forms will be kept in a binder in the flow and imaging core lab.

IV. Pre-Sort Procedures

Individual sorting requests are confirmed by staff prior to appointments, after confirming that all required information has been provided by user and that the information is up to date. Users will receive a confirmation email from the Core Facility Manager if approval is granted. Sort requests without proper information or with any conflicting information will be denied. Users will receive an e-mail about the denied requests and follow-up discussion may be initiated by laboratory staff or user. Only approved sort samples can be delivered to the facility for procedures. ***Sample transportation must strictly follow procedures outlined in the user's biosafety protocol for the appropriate BSL level.*** Samples must be completely processed, stained, and ready-to-acquire prior to sort procedure. Staining procedures, which do not require washing steps (i.e. live/dead stain) may be completed at the flow facility in the biosafety cabinet.

NOTE: All samples must be filtered prior to sorting in order to reduce possibility of clogging the instrument. Samples need to be capped or covered with paraffin before vortexing to minimize aerosols (placing glove over tube is not acceptable).

Flow staff performs the following standard procedure prior to cell sorting on Influx:

- Load 10% volume of house bleach into the waste tank. Ensure that all fluidic tanks are filled.
- Close sort collection chamber.
- Turn on Aerosol Management System (AMS)
- Start the cell sorter and the corresponding computer.
- Perform fluidics start-up and aseptic cleaning when sterile sort conditions are required.
- Turn on lasers.
- Stabilize stream and run daily quality control experiment.
- Stabilize side streams, test sort performance and set drop-delay. Aim sort streams.

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

V. Post-Procedures

After sorting of any material, 10% bleach is run through the cytometer for 5 minutes followed by 5 minutes of dH₂O. The sample injection chamber, collection chamber and the collection device are surface decontaminated with Conflit solution with a contact time of 5 minutes (University of Wisconsin, Madison, n.d.). Computer bench is wiped off with 70% ethanol. The waste tank (containing minimum 10% volume of household bleach) is emptied into sink. The sink is then rinsed with running water for 1 minute. Stream is shut off, lasers are

powered off and appropriate fluidics shut-off procedures are followed. Instrument and computer are turned off. Waste from live samples is decontaminated according to decontamination procedures outlined in the biosafety protocol (see inserted table). All users and staff are required to remove and dispose of any PPE prior to leaving the laboratory. Waste that is deposited in biohazard bags (tubes, tips, other consumables) is autoclaved prior to disposal. All users and staff wash and sanitize hands before leaving the laboratory.

VI. Unexpected Stream Shutoff During Sort Procedure

Clogging and air bubbles may suddenly disintegrate the stream and may result in the deflection of sort streams, spills in the sort collection chamber and significant vapor and aerosol generation. In order to recover the sort stream and re-set, the sort stream needs to be turned off and aerosols need to be evacuated (Turn AMS to 100% for at least 2 minutes) The sorting chamber also needs to be decontaminated (flood collection chamber surface with 10% bleach for 5 minutes, wipe off with towel and then rinse with water) before initiating a new sort.

Material to be Disinfected/Inactivated	Disposal method/Procedure (e.g., autoclave, disinfectant type, or picked up for off-site disposal by MERI or Safety Dept)	Disinfectant concentration and exposure time, or autoclave time and temp
Surfaces (counters and equipment)	Surfaces – 5 min 10% bleach or 5 min "Conflikt" Spray or 70% EtOH BSC interior – 70% EtOH for 5 minutes	10% Bleach or Conflikt 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 30 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 30 minutes
Animal bedding/wastes Other: inside equipment	NA Sorter & Flow Cytometer Tanks	Sheath Fluid Tank – treated thoroughly with 70% EtOH for 20 minutes (tank closed) then rinsed with ddH2O. 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. Spill Procedures

Spill management is done following procedures outlined in our biosafety protocol (see tables 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, left on surface for 5 minutes followed by a water rinse. Large spills on flow cytometer surfaces and inside the sort chamber or on other lab surfaces are cleaned with 10% bleach left on surface for 20 minutes with a soaked, wet towel. 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. Spill kits are provided in the laboratory. 10% bleach

solution (made daily) is provided on all bench-tops. PPE is properly disposed of in biohazard containers after cleaning of a spill.

SPILLS OUTSIDE CONTAINMENT

IMMEDIATE RESPONSE	<p><u>EVACUATE</u> if necessary</p> <ul style="list-style-type: none"> ▪ 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 responders ▪ 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/antimicrobial agent.
CLEAN UP	<ul style="list-style-type: none"> ▪ Wait at least 30 minutes before re-entry to allow aerosols to dissipate. ▪ Wear PPE upon re-entry (disposable gown, mask/eye protection, double gloves). ▪ Cover spill with disinfectant soaked paper towels. ▪ Pour an appropriate disinfectant solution around spill (1:10 dilution of bleach). ▪ Take care not to create aerosols while pouring. Let stand for at least 20 minutes. ▪ Use tools (tongs, broom & dustpan) rather than hands to pick up sharps and broken glass, and contaminated materials whenever possible. ▪ Transfer all contaminated materials into an autoclave bag. ▪ Wipe down entire area with disinfectant (including autoclave bags, disinfectant container and other contact surfaces).
WRAP UP	<ul style="list-style-type: none"> ▪ Remove and discard PPE (autoclave prior to disposal). ▪ Shower or wash hands with soap/antimicrobial agent and water. ▪ Autoclave all contaminated materials. ▪ Report incident to the PI (if not already contacted), the BSO (Biological Safety Officer) and Occupational Health

SPILLS INSIDE CONTAINMENT

INITIAL RESPONSE	<ul style="list-style-type: none"> ▪ <u>Immediately stop all work, but leave BSC or hood blower fan on during clean-up.</u>
CLEAN UP RESPONSE	<ul style="list-style-type: none"> ▪ Wear PPE (gloves, lab coat, eye protection). ▪ Cover spill with disinfectant soaked paper towels and pour an appropriate disinfectant solution around spill. ▪ Using paper towels and appropriate disinfectant detergent, wipe down walls, work surfaces and equipment. ▪ Flood work surface and drain pan (Type II BSC) with disinfectant and let stand for at least 20 minutes. ▪ Wipe up all excess disinfectant and empty drain pan into a collection vessel with disinfectant. ▪ Flush drain pan with water and remove drain tube. ▪ Transfer all contaminated materials into an autoclave bag. ▪ Wipe down autoclave bag, disinfectant container and other contact surfaces with disinfectant.
WRAP UP	<ul style="list-style-type: none"> ▪ Remove and discard PPE (autoclave prior to disposal). ▪ Wash hands with soap/antimicrobial agent and water. ▪ Autoclave all contaminated materials. ▪ Report incident to the PI (if not already contacted).

VIII. Waste Management

Empty flow cytometer waste tanks are filled with 10% volume of household bleach prior to pouring down the sink as a standard facility procedure (waste is treated for at least 30 minutes otherwise). Content is emptied into sink and sink is rinsed for 1 minute.

Liquid waste in sample tubes if smaller than 1 ml total volume can be re-capped and disposed of in the biohazard bags for autoclaving. Any larger volumes of liquid waste needs to be treated with 10% bleach for 30 minutes prior to disposal in the sink followed by a 1 minute rinse. Solid waste is disposed in biohazard bags and autoclaved as described in the Decontamination Table on Page 3. Solid decontaminated waste can be disposed of in our biosafety hazard bags for autoclaving only, if proper procedures outlined in this SOP are followed.

IX. Aerosol Management System

The Aerosol Management System installed on the Influx Cell sorter 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 Ultra-Low Penetrating Air filter is replaced after the indicator light is blinking or after 6 months. The Protection-Plus Prefilter will be changed monthly.

X. Operator Training and Experience

Core facility personnel are the only individuals allowed to perform sorts using the Influx cell sorter. Core facility users are not allowed to operate the Influx cell sorter but will be provided with copies of the SOP and instrument protocols at the initial training. Copies are also available in the laboratory and online on our core lab website.

(<http://research.ouhsc.edu/core-facilities/flow-cytometry-and-imaging-2/standard-operating-procedures/>)

Training for the user on this instrument includes basic instrument operation and all relevant safety procedures, spill management, and decontamination so that they understand what the operator is doing while running their samples.

XI. Exposure to Biohazardous Material

All employees with occupational exposure should receive bloodborne pathogen training 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. These employees should also take the online Bloodborne Pathogens training course annually (found on the EHSO website). 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); and
- 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)

1. Flow Cytometry Core Facility, Carbone Cancer Center, University of Wisconsin, Madison. "Standard Operating Procedures: Safe Working Practices for Cell Sorting and Live Cell Benchtop Flow Cytometry." (n.d.): n. pag. Flow Cytometry Core Facility, Carbone Cancer Center, University of Wisconsin, Madison. Web. 21 Sept. 2015. file://localhost/<http://www.uwhealth.org/files/uwhealth/docs/cancer_for_researchers:UWCCC_Flow_SOP.pdf>.

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 Through Friday and weekends