

Standard Operating Procedure (SOP) for BSL2+ FACS Aria Cell Sorter

Flow Cytometry and Cell Sorting (FCCS) Shared Facility Hollings Cancer Center (HCC), Medical University of South Carolina (MUSC)

1. Purpose

The purpose of this procedure is to outline a safety plan for receiving and processing samples using a Becton Dickinson (BD) Biosciences FACS Aria cell sorter under BSL2+ conditions in the HCC FCCS Shared Facility.

2. Background

The HCC/MUSC FCCS Shared Facility is a HCC and MUSC Core Facility, and co-sponsored by HCC, the University Administration (Office of the Vice President for Academic Affairs and Provost), and the SC COBRE for Oral Health and the Center for Oral Health Research. The facility provides analytical flow cytometry services and sorting services which are essential for *in vivo* and *in vitro* experiments for many investigators at HCC and MUSC. The facility is centrally located on the first floor of the Basic Science Building. The sorting facility is located in BSB-129A and the analytical facility is located in BSB-129C.

BSB-129A has a dedicated air handling system and excellent negative air flow to prevent recirculation of aerosolized pathogens.

The process of high-speed sorting can produce aerosols that may constitute a health hazard to facility personnel. The safety of the staff and users of the facility is the ultimate concern. The procedures below are designed to protect the operator of the cell sorter under high-speed sorting conditions.

3. Introduction

The BSL-2+ FACS Sorting involves moderate to high-risk agents and therefore requires a strict adherence to BSL-2 containment with BSL-3 work practices and procedures (i.e. BSL2+). It is important that all personnel that work in the BSL-2+ facility understand and adhere to the proper procedures and techniques outlined in this manual. Failure to adhere to appropriate practices and procedures may endanger others. All the people who are going to use the BSL2+ FACS Sorter should also follow the procedure of SOP for BSL2+ lab. Due to the complex nature of the FACS Aria and cell sorting, this instrument will only be operated by the core manager (Rick Pepler) at the present.

3.1 Agents to be used in the BSL-2+ FACS Sorter.

A. Unfixed human cells from patient blood, body fluid, or other unfixed tissues obtained from patients not thought to be infected with any >risk group 2.

- B. Murine or human cells which have been infected with recombinant retroviral or lentiviral vectors which have been IBC-approved for \leq BSL-2 containment.
- C. Murine or human cells which may have been exposed to bacteria from Risk Group 2 or lower.

Samples known to contain BSL3 pathogens and some BSL2 pathogens (considered as BSL3 in practice as described in “Biosafety Guidelines for Sorting Unfixed Cells”, Ingrid Schmid et al, Cytometry 28:99-117 (1997) will not be accepted for sorting. These include Hepatitis viruses B and C, HIV-1, HIV-2, HTLV-1, HTLV-2, tuberculin bacillus, Herpesvirus Simiae (Herpes B virus), and SIV (Simian Immune deficiency virus). The facility director and manager will make decisions concerning sorting of other known infectious agents on an individual basis in consultation with the Biosafety Officer.

To assure the assignment of proper safety practices and procedures, it is necessary to collect detailed information about all samples prior to receipt. A Sorting Information Sheet and Biohazard Sort Form (see attached forms) must be completed and submitted to the Flow Cytometry Facility Manager at least 48 hours prior to the first sort of each project. The form will be reviewed by the core manager and the Occupational Safety and Health Program (OSHP) Biosafety Officer, and an appropriate containment will be assigned based on risk.

Samples must be properly packaged prior to transport to the facility. Failure to comply with the following packaging requirement may lead to the rejection of the sample:

Samples must be contained in a leak proof container and clearly labeled with a sample identifier and a biohazard symbol. Samples should be transported on campus in sealed leak-proof primary and secondary containment. The secondary (outer) container should have the biohazard sticker. Samples could be provided in sealed or capped tubes placed in larger conicals, sealed specimen bags, Tupperware or test tube racks placed inside of a larger sealed container.

Sample must be clump free and in an appropriate tube for sorting (12x75mm snap-cap tube or 15ml conical tube). Filtration of samples can create splashes or aerosols. If samples require filtration to remove clumps, this should be done either in a biosafety cabinet or in the BSL2+ facility with appropriate PPE and respiratory protection.

Sample must not contaminate the outside of the tube.

3.2 Cell Sorting Facility and Facility Access

3.2.1 Facility

FACSAria Sorter is located in BSB-129A. There are two doors that separate this room from the corridor and the pressure differential across each must be

progressively more negative as one enters the facility. As a daily routine, we will use a smoke test device to ensure that the facility is under negative pressure. If the facility is not under negative pressure, no BSL2+ sorting can be performed. We will need to contact Engineering & Facilities maintenance (HVAC Shop) at 792-4119 to resolve the issue.

The OSHP office will perform the initial testing of the facility to certify constant negative air pressure in the FACS core. The operator of the facility will be required to perform daily qualitative checks of negative air pressure with a simple smoke test at the entrance to the facility. The kit to perform this test is available at the following link: <http://www.labsafety.com/search/smoke+kit/16730/89496/>

3.2.2 Warning Signage

A universal biohazard warning sign shall be posted at the doorway to the sorting facility. The sign will indicate the biosafety level and the types of hazards in the lab. A second sign shall be posted to communicate entry and exit requirements for the facility when active work is being conducted.

3.2.3 Laboratory Entrance Requirements

Pay attention to all warning signs posted on the door. Access to the facility is restricted to those persons whose presence is required for experimental or support purposes. The entry door is locked when sorting is in progress and during non-working hours. The Facility Director and Manager have the final responsibility of assessing each circumstance and deciding who may enter the facility.

Laboratory Personnel - Put on the required PPE (see PPE section) upon entry into room BSB-129A. If an active sort is in progress, contact the operator running the sort by intercom to inquire when it is appropriate to enter the room. If entrance is deemed necessary, the sort will be paused before entering the room.

Visitors - Other individuals permitted to enter the laboratory when sorting is not in progress include personnel from Housekeeping who remove waste and clean the laboratory, and from Facilities Management to repair and maintain utilities.

Contractors and Vendors - Field service engineers from Becton Dickinson Immunocytometry System are allowed to enter the facility to service the instruments except when BSL2 sorting is in progress.

3.3 Medical Surveillance

Medical surveillance is performed through Employee Health. All research personnel that work in the Flow Cytometry Facility must complete the following through OSHP:

Training in Bloodborne Pathogens, Chemical Hygiene, and Hazardous Materials (this training must be renewed annually);

Hepatitis B vaccination (must sign waiver if refused);

Occupants of the facility must be fit-tested with a N95 mask by OSHP;

The cell sorter operator goes go to Employee Health for baseline screening of BBP. This will be repeated as part of his/her annual employee physical examination.

Procedures for accidental exposures:

a. TREATMENT OF EXPOSURE

Open wounds: Soap & Water x 5", saline irrigation if available

Mucous membrane: Flush extensively with water x 5-10"

b. REPORT THE EXPOSURE

Employee Health Services (EHS) at 792-2991*

If after hours, call 792-2123 to page the HSC (Hospital Service Coordinator).

Inform EHS/HSC if source is positive for HIV or other pathogens

EHS will fax a Source Patient Lab Requisition form

c. LABORATORY TESTING

Have blood drawn either at EHS or Rutledge Tower

Draw 2 small or 1 lg SST tube and 1 small lavender tube
(no microtainer tubes)

Label with pt stamp plate

Place in specimen bag with requisition

d. TRANSPORT

Specimen to Children's Hospital Room 319 Specimen Receiving (792-0707).

e. GO

To EHS (57 Bee Street)

Bring patient information (Name, MRN, pertinent medical history) & completed ACORD form available online at

http://mcintranet.musc.edu/ehs/work_related.htm

f. EHS will

Assess, follow-up on lab results, and counsel employee

NEEDLESTICK HOTLINE 792-4422 effective 11/03/03

g. Notify the Biosafety Officer at 792-4304 (eisenman@musc.edu) to report exposures to human materials, infectious agents or recombinant DNA.

4. Standard Laboratory Practice

Eating, drinking, smoking, handling of contact lens, and applying cosmetics are absolutely prohibited in the facility.

Mouth pipeting is prohibited.

Policies for the safe handling of sharps are required.

Sandals or any open toed shoes are prohibited. Shoe covers should be worn when aerosols, splashing or spills are likely.

The use of disposable lab coats is recommended but the use of currently approved cloth lab coats can also be worn. Cloth coats must be laundered by the employer and never taken home.

A signage of “sorting in progress” will be placed on the door during the sort.

All work surfaces are decontaminated once a day and after any spill of viable material with freshly prepared 10% bleach for 15 min, wiping down the residual, and then rinsing with 70% ethanol. During special procedures areas should be decontaminated prior to working in area.

Facility doors are kept closed and locked during all experiments in progress.

All cell cultures, stocks and other regulated wastes are decontaminated by autoclave sterilization before disposal as Medical Pathological Waste (MPW).

5. Personnel Protective Equipment (PPE)

5.1 Entering the Facility

Lab coat, gloves and close-toed shoes are the minimal PPE required for work in the laboratory under BSL1 conditions. Such PPE would be appropriate for work with unfixed cells.

PPE requirements upon entry and while sorting any unfixed sample should include the following:

Seamless-front gown with cuffs

Full-face protection

Double-gloves

Close-toed shoes

Respiratory protection

Shoe covers

All persons in the BSL2+ FACS Sorter facility should wear BSL-2+ PPE. Any open wounds should first be covered with occlusive bandages prior to donning PPE. Gloves should be donned first, followed by respiratory protection (N95 mask or respirator), then eye protection (safety glasses, goggles or plexiglass face

shield), and last, a lab coat or disposable lab gowns (preferably with a knit or grip cuff). A second pair of gloves should be donned, extending over the sleeve of the lab coat to avoid allowing any gaps between the lab coat and glove to ensure proper wrist coverage.

When sorting samples that are known to contain pathogens and that pose an inhalation risk, additional or alternate respiratory protection may be required. In such cases, the operator may need to don a powered air-purifying respirator (PAPR).

5.2 Exiting the Facility

The outer pair of gloves should be removed first before exiting the facility. These should be immediately disposed in the red biohazard waste box near the door to the BSL-2+ facility (BSB-129A).

Don a new pair of gloves over the inner pair.

Disposable lab gowns will be disposed into a red biohazard waste box placed next to the door into the facility for this purpose. Remove the outer pair of gloves and dispose in the red biohazard waste box.

Remove respiratory protection. If disposable, like an N-95 dust mask or respirator, dispose in red biohazard box. If non-disposable, decontaminate by spraying respirator with freshly prepared 10% bleach, wiping down the residual, then rinsing with 70% ethanol. Non-disposable respirators should remain in BSB-129A for future use. This operation should be performed over the blue chux on the counter adjacent to the sink in BSB-129A and all waste paper towels should be disposed in the red biohazard waste box.

Eye protection should be removed and decontaminated by spraying with fresh 10% bleach solution, wiping off residual, and then rinsing with 70% ethanol. This operation should be performed over the blue chux on the counter adjacent to the sink in BSB-129A. All waste paper towels should be disposed in the red biohazard box.

Dispose of blue chux in the red biohazard box.

Remove the final pair of gloves worn during FACS sorting and discard into the red biohazard box in BSB-129A.

Before exiting the sorting facility, hands should be thoroughly washed (2 minutes) with an antibacterial soap and warm water.

6. Bio-Containment and Infectious Cell Sorting

The agents described in Section 3.1 will be run through a high-speed cell sorter (BD FACSAria), which is capable of generating aerosol under normal operating conditions. Thus, proper aerosol management should be in place. FACSAria is equipped with aerosol management system (AMS). However, it is the operator's responsibility that the AMS must be on and functioning according to the manufacturer guidelines.

6.1 Aerosol Management System (AMS) of FACSAria

- A. Appendix A shows the aerosol flow and the locations of the vacuum gauge and monitor. The vacuum monitor should be set to **20%** and the vacuum gauge must read between **1.0 and 1.5 inches of HoH**. If it is outside of this range, check to make sure that the filter is seated correctly. If gauge does not come into range replace HEPA filter unit and tubing.
- B. The sheath waste tank must contain enough bleach to provide a final concentration of 10% when filled (1L beach to a final 10L waste collected). The sheath waste tank will be emptied at the end of each sort. Fresh 10% bleach will be added at the beginning of each sorting procedure. It is the responsibility of the individual users to discard and refill the bleach in the waste container after each sort. Failure to adhere to this practice may result in suspension of access privileges to this facility.
- C. The Accudrop camera system must be functioning normally according to the manufacturer guidelines. This camera system is used to monitor the sort stream and alerts the operator to potential increased aerosols. In this situation the operator can correct the sort stream and reduce aerosol contamination. The FACSAria is equipped with a Sweet Spot, which is used during all sorting operations. The (Sweet Spot) device detects stream drifts as well as possible clogs in the system and will automatically shut down the stream if a clog is detected.

6.2 Measurement of Containment and Tolerances

- A. The **Aerosol Management System AMS** must be tested under simulated worst-case failure mode. In this mode the instrument is set to 70 psi and 50,000 particles/second, with the waste catcher blocked to create large aerosol. This is done by covering waste catcher with a cut off piece of sample tubing (Appendix B).
- B. Add glass slide to the AeroTech concentrator and place directly on top of sort chamber (Appendix C and Appendix D). Adjust the pressure to the AeroTech concentrator to 55 LPM (liters per minute). Close door to deflection plates but do not install tube holders. The main sort chamber should also be closed and the AeroTech device should be placed directly on top of this chamber door.
- C. Turn AMS on (20%) and check for proper vacuum function (1.0-1.5 inches of HOH).
- D. Place Glo-Germ particles (5-mm melamine copolymer resin beads in a 5-ml volume of ethanol) onto the sample station and adjust either the particle concentration or the flow rate to achieve a particle rate of 50,000 particles per second. Note: When creating aerosols, which could contain Glo-Germ

particles, it is recommended the operator wear a 0.2 μ m particle mask. Glo-Germ powder can be purchased online at: <http://www.glogerm.com/>.

- E. Begin acquiring Glo-Germ particles and allow collection for 5 minutes. (see Appendix E).
- F. Turn off vacuum to AeroTech concentrator and remove slide from inside. Put in a fresh slide and continue collecting aerosol with the AMS turned off for another 5 minutes (positive control). Stop sample acquisition and remove waste catcher plastic sleeve.
- G. Examine glass slides for bright green fluorescence using a fluorescent microscope equipped with a FITC filter (520-640nm). Glo-Germ particles will form a pattern as directed by the holes found in the plate cover (see Appendix F). Therefore, when present, the location of the particles can be found in this pattern.
- H. Scan the entire slide on 10x and count all Glo-Germ particles. The positive control slide can be used as a reference to distinguish between fluorescent debris and actual Glo-Germ particles. Record all data.
- I. Acceptable Tolerance: No Glo-Germ particles detected after 5 minutes of active air sampling in front of sort chamber door with no tube holder in place and the AMS turned on. The positive control slide must contain greater than 100 particles after 5 minutes of active air sampling with the AMS turned off and no tube holder in place.

The flow cytometer must pass all tolerances of aerosol containment as described in this procedure. If these tolerances are not met, infectious cell sorting is not permitted.

6.3 Routine Sorting Procedure

Upon entering the BSL2+ Sorter room, the operators must wear personnel protection equipment (PPE) as outlined in Section 5. If the operator is not protected as described in this section, infectious cell sorting is not permitted.

The room is limited to 2-3 individuals during the sort procedure. All must adhere to the highest level of protective practices (PPE, etc.) required for the material/operations within the room. All must be adequately informed of the risks of working with all agents within the room and the potential consequences of exposure to these agents.

Turn on the FACS sorter and verify that the AMS is working correctly. This device must have a vacuum pressure of 1.0-1.5 SCFM. If this tolerance is not met, infectious cell sorting is not permitted.

Make sure the sheath tank is full.

Empty the waste tank into the only after the waste liquid has been exposed to 10% bleach for at least 30 minutes. Pour concentrated bleach into the waste tank making sure that there is about 10% bleach when the tank is full.

Close all barriers around the sort chamber. If this is not done, infectious cell sorting is not permitted.

QC the instrument with manufacturer recommended alignment bead to make sure laser timing and area scatter are within recommended tolerances.

The stability of the sort side streams and droplet break-off shall be tested prior to each sort with the aid of the AccuDrop management system.

All samples for sorting must be filtered through a 35 μ m mesh prior to sorting. This reduces the potential for clogging and decreases the risk of generating aerosols.

Start sort and monitor the sort performance using the AccuDrop camera. If during the sort the stream is deflected (due in part to a clogged flow cell tip), the sorter is designed to stop automatically and block the sort tubes. The sort will not restart until the operator has cleared the clog. Use the following procedure to remove a clog from the Cytometer:

- a. Remove the sample from the sample chamber.
- b. Turn stream off and then on again to see if drop delay and stream returns to normal pattern. If clog is not fixed select the nozzle flush procedure and use Contrad 70.
- c. If the nozzle cannot be cleared, the system should be shut down and allowed 5 minutes to reduce the potential inhalation of aerosols generated during the sorting process.
- d. Be certain that the high voltage deflection plates are turned off (view red light).
- e. Remove the sort sample, open the collection chamber and remove the collection vials.
- f. Remove the nozzle and clean with 10% bleach solution for 2 minutes, then placed it in a 50 ml centrifuge tube containing Contrad 70 solution. The nozzle is then cleaned for 2-5 minutes in a mechanical sonicator.
- g. After replacing the nozzle, gloves used for cleaning the clog will also be discarded and fresh gloves will be applied. The area around the FACS sorter will wiped with fresh 10% bleach followed by 70% ethanol until nearly dry.
- h. Sorting can be resumed after the flow cell tip is cleared making sure that the stream is stable, and the droplet break-off and side streams are stable.

After sort is completed, samples can be removed immediately after sample tube is unloaded from the sample station.

When sort is finished, 10% bleach solution will be run through the sample lines for 2 minutes and the inside and outside of the sort chamber will be sprayed and wiped down with 70% ethanol.

Areas around the FACS sorter will be wiped first with a fresh 10% bleach solution then 70% ethanol to eliminate any potentially aerosolized material that may have been generated, released and settled on the benchtop at the completion of the sorting. All materials generated in the process of the sorting procedure or in the shutdown and cleaning procedure will be disposed of in the Red Biohazard waste containers.

6.4 Routine Maintenance

The FACSaria is under an annual service contract and receives two quality control service calls per year. Fluidic lines are checked daily for leaks or crimping. Quality control beads are run daily to make sure the stream, break-off, and lasers fall within recommended tolerances. All AMS and sort chamber filters are monitored to insure proper operation. Any deviation beyond these tolerances and the sort is terminated.

7. Waste Management

Decontamination of waste is the responsibility of each user and must be completed before exit from the BSL-2+ facility.

7.1 Liquid Waste

All liquid wastes must be collected and decontaminated by diluting with fresh bleach to a final bleach concentration of 10% (0.5% sodium hypochlorite) for at least 30 minutes prior to disposal down the sink in BSB-129A, flushing the sink with copious water. Liquid waste should not contain any other chemicals which cannot be disposed into the sewer system and are not permitted in the facility, unless waste disposal methods have been specifically authorized by the IBC and Chemical Safety Office prior to use.

7.2 Solid Waste

All solid waste shall be collected in a biohazard bag, the exception being pipets or other sharps. Use of sharps (needles, syringes, glass) should be discouraged in the BSL-2+ facility. All efforts should be taken to identify alternatives to glass or sharp alternatives. If no alternatives to sharps are available, sharps shall be placed in puncture-proof containers prior to placement into biohazard bags. Any needles used should be disposable "safety needles" that are deposited in an appropriate sharps container, which will be disposed of in a biohazard bag. Filled bags will be loosely closed with temperature indicator tape. The waste will be transported to the autoclave room located in BSB-215 via the freight elevator. Transportation of the waste must be done in a leak-proof container. The waste will be autoclave for 45 minutes at 121°C.

8. Spills & Decontamination Procedures

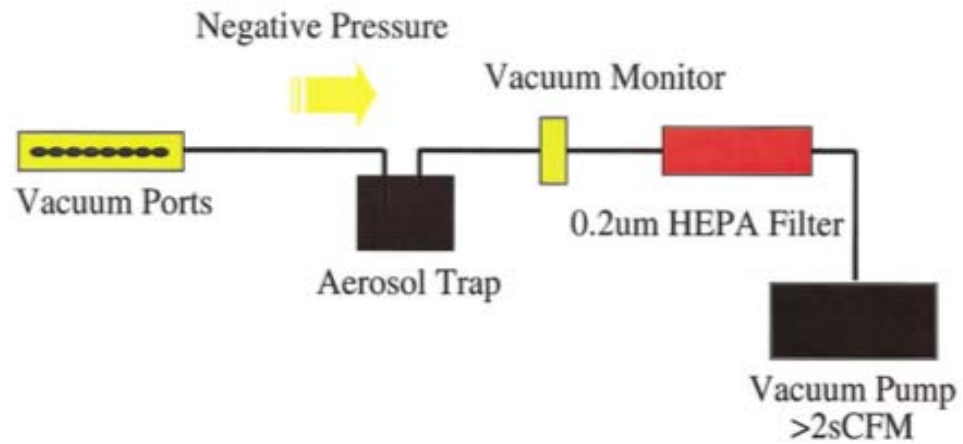
Spills of biological materials are decontaminated, contained, and cleaned up by the core manager (Rick Pepler), or other properly trained and equipped to work with concentrated infectious or potentially infectious material. Spills and accidents that result in overt exposures to BSL2+ materials are immediately reported to the Facility Director or Manager. Appropriate medical evaluation, surveillance and treatment are provided by the MUSC Employee Health Services and written records are maintained.

Inside Biosafety Cabinet (BSC): Allow 5 minutes for the BSC to contain aerosols. Remove any contaminated clothing and change gloves. If needed, use the emergency shower and/or eye wash station located by the sink. Place a dry paper towel on the liquid and place a second application of disinfect-soaked paper towel over the spill. Surround the spill with disinfectant solution, cover with paper towels, and let sit for 15 minutes. Wipe the walls, work surface and any equipment in the cabinet with a disinfectant-soaked paper towel. Dispose of all contaminated waste in appropriate biohazard containment bags to be autoclaved.

Outside Biosafety Cabinet: Evacuate the area and close all doors. Wait at least 15 minutes for the aerosols to settle before entering the area. Remove any contaminated clothing and change gloves. If needed, use the emergency shower and/or eye wash station located by the sink. Place a dry paper towel on the liquid and place a second application of disinfect-soaked paper towel over the spill. Surround the spill a disinfectant solution and allow 15 minutes of contact time. Wipe equipment with appropriate disinfectant. Dispose of all contaminated waste in appropriate biohazard containment bags to be autoclaved.

Appendix

A. Aerosol Management System (AMS)



B. Aerosol formation in sort block



Standby mode



Active mode

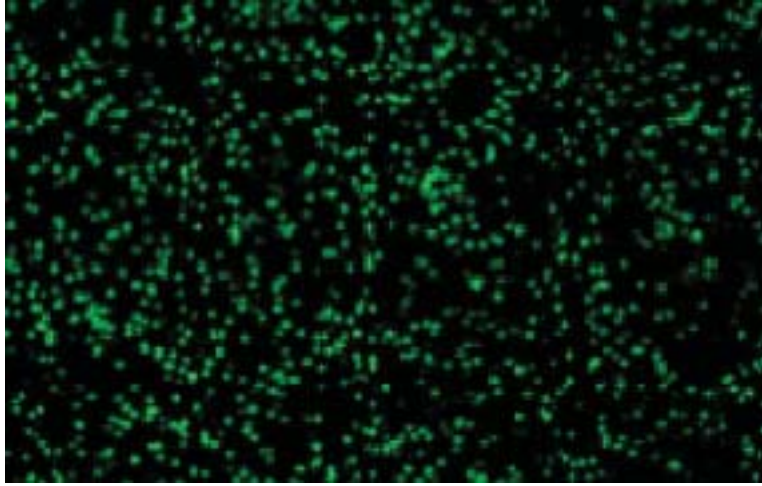
C. AeroTech collection device



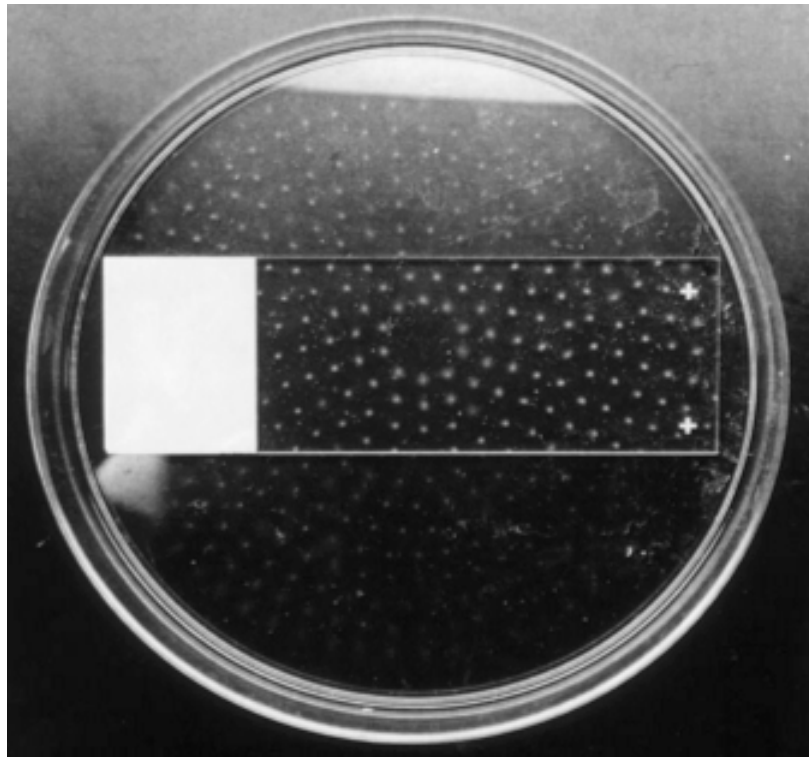
D. Aerotech device on sort chamber

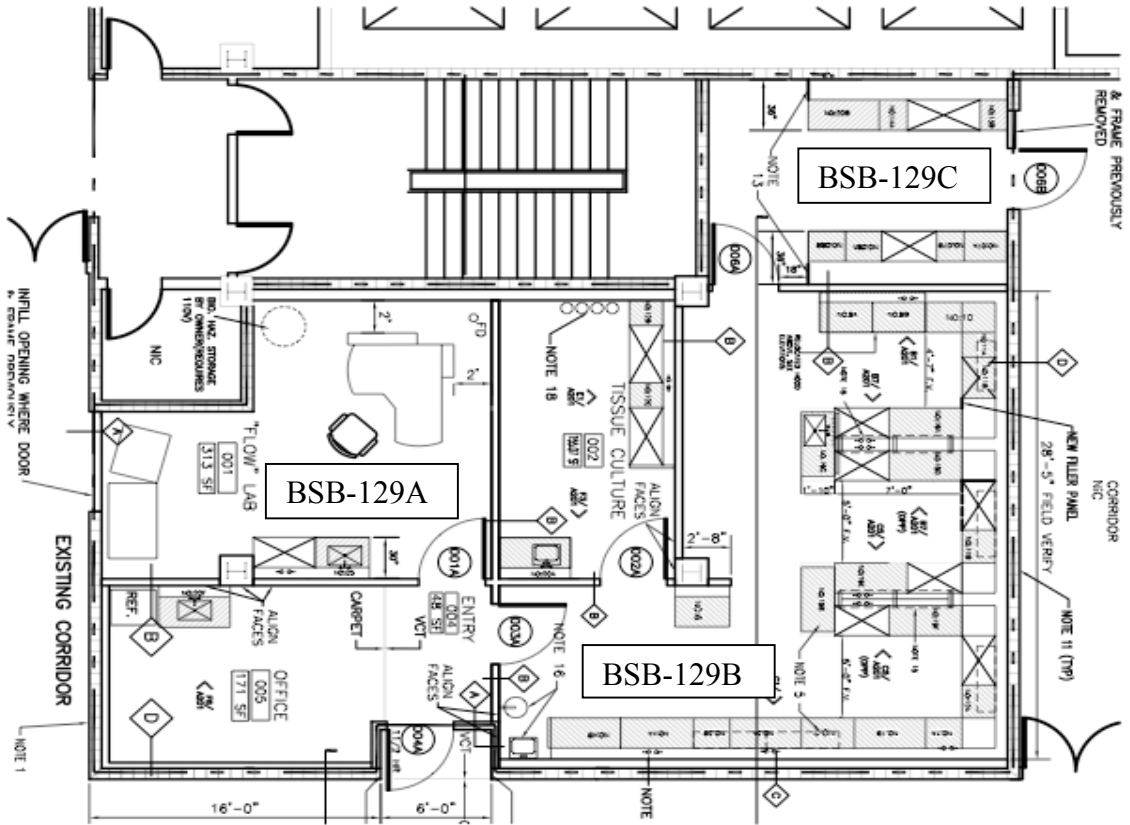


E. GloGerms particles under fluorescent microscope



F.





MUSC Flow Cytometry Facility
Sorting Information Sheet

User: _____

Date: _____

E-mail: _____

Phone: _____

PI: _____

Cost Center # _____

Experimental Goal of Sort:

Cells to be Sorted:

(Please use one form for each sample or type of cells to be sorted, unless samples are similar and have same sorting parameters)

Cell type: Cell Line Primary Cells Other: _____

Species: Human Mouse Rat Other: _____

Tissue Origin: Bone Marrow Peripheral Blood Neural Other: _____

Adhesiveness: Non-Adherent Cell Line Adherent Cell Line
 Non-Adherent Tissue Disaggregated tissue

Mode of Disaggregation: Trituration Trypsin Collagenase Other: _____

Estimated Cell Size: < 10 μm 10-20 μm > 20 μm

Number of starting cells (#): _____

Volume of starting cells (ml): _____

Concentration of starting cells (#/ml): _____

Carrier Solution: PBS HEPES Hanks BSA ___% FCS ___%
 Other: _____

Is the sample sterile? Yes No Does the sort need to be sterile? Yes No

Is the sample filtered? Yes No

Sorting Parameters:

488 nm Laser

Blue A (785/60 nm): PE-Cy7
Blue B (695/40 nm): PE-Cy5.5 PerCP-Cy5.5 7AAD
Blue C (610/20 nm): PE-Texas Red
Blue D (575/26 nm): PE PI
Blue E (530/30 nm): FITC Alexa Fluor 488 DCF GFP

633 nm Laser

Red A (780/60 nm): APC-Cy7
Red B (660/20 nm): APC Alexa Fluor 647

407 nm Laser

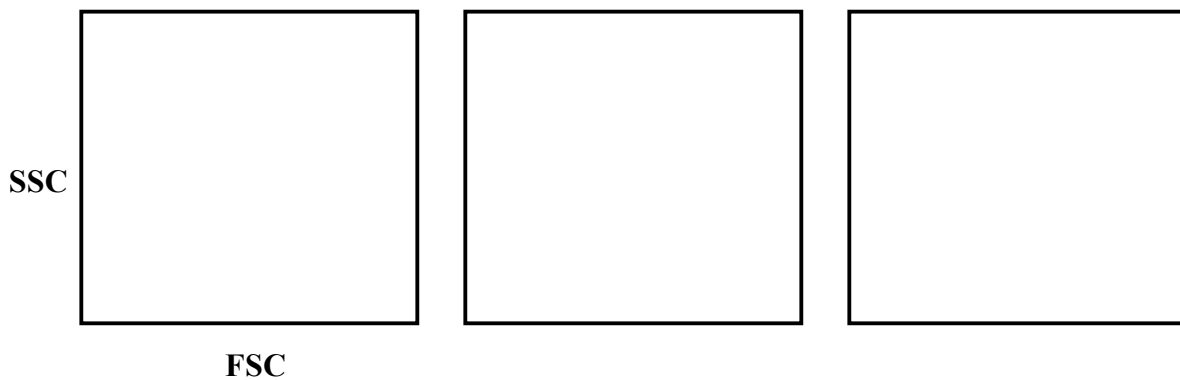
Violet A (530/30 nm): Alexa Fluor 430 Qdot 520
Violet B (450/40 nm): Cascade Blue Pacific Blue DAPI
 Hoechst Alexa Fluor 405

If your fluorochrome or dye is not listed, please indicate below.

Name: _____ Excitation: _____ Emission: _____

Sorting Schema:

(Please show Fluorescent patterns, gating parameters and hierarchy of target cells and subsets.)



Target Subsets:

(Indicate Fluorochromes positivity and negativity, e.g. FITC+ APC-, and show target subsets in the plots above.)

	~% cells in sample	~# cells needed after sort
<input type="checkbox"/> Subset 1:	_____ %	_____ %
<input type="checkbox"/> Subset 2:	_____ %	_____ %
<input type="checkbox"/> Subset 3:	_____ %	_____ %
<input type="checkbox"/> Subset 4:	_____ %	_____ %

Re-analysis of target cells after sort? Yes No

Collection Method:

Temperature Setting: 4°C 15°C Room Temp 37°C Other: _____

Sort Speed: Low (20 PSI) Medium (35 PSI) High (70 PSI)

Collection Device: Analysis Only
 2-way sort: 1 ml tubes 15 ml tubes
 4-way sort: 1 ml tubes
 Plate: 6-well 12-well 24-well 48-well 96-well
 Slide

For Operator Use Only:

Scheduled date and time of sort: _____

Actual start time of sort: _____ Finish time of sort: _____

Number of hours (not counting setup time):

1 hour 1.5 hours 2 hours 2.5 hours 3 hours Other: _____

Billing: Yes Date of billing: _____

Result of sort:

Approximate number of sorted cells (estimation).

Subset 1 _____; Subset 2 _____; Subset 3 _____; Subset 4 _____

Successful sort, no problems encountered

Clumping of cells experienced

Low yield

Purity of sorted cells question

Cells might be bacterially contaminated

Technical problems with sorter

Other Comments:

Biohazard Sort Form

MUSC Flow Cytometry Facility is a multi-user facility where many different samples from various sources that contain unknown animal and human pathogens are analyzed or sorted. The safety of the staff and users of the facility is the ultimate concern. Therefore, information about the sample sources and infectious agents is critical for effective biosafety risk assessment.

This form must be filled out completely and signed by the principal investigator. The form will then be reviewed by the IBC before experiments are started. Appropriate biosafety approval prior to use of the facility is required. Failure to obtain approval may jeopardize future use of the facility.

The process of high-speed sorting produces aerosols that can constitute a health hazard to facility personnel. For this reason the sample restrictions listed below will be followed:

- **Primary human materials must have documented proof of HIV, hepatitis B and hepatitis C negativity prior to sorting.**
- **No samples containing radioactivity will be sorted.**
- **No bacteria or yeast will be sorted.**

Sample biosafety level: BSL1 BSL2 BSL3

Are these samples of human origin? Yes No

If yes, were the donors screened for bloodborne pathogens? Yes No

Has the infectious agent been inactivated? Yes No If yes, describe the inactivation method.

Were the cells transformed using a virus such as EBV, HTLV-1, herpes saimrii, etc? Yes No If yes, list virus.

Are there exogenous genes transferred into the cells to be sorted: Yes No

If "yes", list the genes here: _____

Are any of these genes oncogenes or pathogenic genes? Yes No

Method of gene transfer:

Chemical or Physical Method: Ca Phosphate Lipofusion Electroporation

Viral Vector: Adenovirus Retrovirus Lentivirus Other

Name of Vector: _____ Source of Vector: _____

Name of packaging cell line: _____

The viral preparation has been shown to be free of replication-competent virus:

Yes No

Can the original viral vector used infect human cells?

Yes No

Does the PI have IBC approval to possess and use the cells and/or virus? Yes No

All requests for sorts involving BSL-2 level organisms and/or infected samples requiring IBC approval will be verified. Please supply a copy of your IBC approval when submitting this form. If the PI does not have approval from the IBC for possession of the cells and/or pathogen, the sort time will be canceled.

Principal Investigator Signature _____

Operator: _____ Date: _____

11-02-2005