Preface to NIH Biosafety Policy for Cell Sorters

The attached document provides Biosafety Policy for the NIH Intramural community for the installation and operation of the class of instruments known as cell sorters, or fluorescent activated cell sorters.

For existing cell sorter laboratories, the following timeline for implementation is to be adopted:

1) All cell sorters on NIH facilities shall be equipped with an aerosol management system within 1 year from approval of this document by the Deputy Director of Intramural Research (NIH/DDIR).
2) For laboratories performing cell sorting experiments on samples/agents classified as BSL2 with enhanced precautions, within 3 years of approval of this document by the Deputy Director of Intramural Research (NIH/DDIR) either:
    a. Cell sorters are to be located within a separate lab space and policy adopted for PPE, including respirator use for the operator as outlined in Section 6.

    OR

    b. Cell Sorters are to be placed within a Class II BSC and policy for PPE, including respirator use for the operator as outlined in Section 6 are optional but strongly encouraged.

For all new installations of cell sorters beginning in FY '12, this policy must be immediately adopted.

NIH Biosafety Policy for Cell Sorters

Introduction

The purpose of this document is to provide biosafety practices for the installation and use of instrumentation known as fluorescence activated cell sorters. By design, stream-in–air cell sorters produce aerosols and therefore use of this instrumentation with infectious or potentially infectious samples defines this process as a procedure hazard.

A fundamental objective of any biosafety program should be containment and the determination of procedures designed to reduce exposure based upon a thorough risk assessment. This Policy is meant to reduce or eliminate exposure of the outside environment and laboratory personnel to potentially hazardous agents during the operation of cell sorters and aimed at providing:

1) Guidance for the design of laboratories housing cell sorters
2) Guidance for the creation of laboratory or instrument specific Standard Operating Procedures (SOP).
3) Guidance on procedures for the safe operation of cell sorters and validation of their aerosol containment systems

This Policy is derived from established biosafety principles as outlined in the BMBL((http://www.cdc.gov/od/OHS/biosfty/bmbl5/bmbl5toc.htm) and the current International Society for the Advancement of Cytometry (ISAC) Biosafety Standards (http://www.isac-net.org/index.php?option=com_content&task=view&id=660&Itemid=46) .

This Policy supplements other safety policies at the National Institutes of Health and does not reduce or alter the requirements of any other policies.

Definitions

The class of instrumentation known as flow cytometers consists of analyzers and cell sorters. Analyzers are designed to measure light scatter and fluorescence of cells or particles in a fluid stream in a closed system and therefore are unlikely to produce aerosols. Cell sorters also perform analysis of cells or particles, but are designed so that desired cells are isolated into droplets that are deflected electrostatically into open collection vessels. These cell sorters are known as stream-in-air or jet-in-air cell sorters, which describes the ejection of fluid from the

nozzle into the open air. The likelihood of aerosol production by cell sorters is high due to the possibility of fluid exiting a small orifice (usually 70µm) at high pressure (up to 70psi) impacting a hard surface (1). Aerosol production is highest in the event of a partial obstruction of the nozzle orifice and subsequent stream deviation. There are some models of flow cytometers that also provide cell sorting capability via a different mechanism. These cytometers utilize a piezo-driven sorter mechanism together with a closed fluidic system, in which the risk of aerosol release is minimal. Two examples of this type of sorter are the FACS Calibur (BD Biosciences) and the PPCS (Partec). This type of piezo-driven cell sorter is exempt from this policy.

Risk Assessment

The purpose of Risk Assessment is to recognize and identify hazards and measure the risk or probability that something will happen because of that hazard. The results of a comprehensive risk assessment determine the appropriate procedures and practices for cell sorting. The designation of safety measures is dependent upon the risk and the severity of the consequences if exposure occurs. Risk analysis takes into account the risk group of the agent and the procedures performed with the agent.

Risk assessment is comprised of five steps:

1. Identify and evaluate agent hazards
2. Identify laboratory procedure hazards
3. Make final determination of biosafety level
4. Evaluate proficiencies of staff and integrity of safety equipment
5. Review risk assessment with biosafety professional

The risk group of the agent can be determined from a variety of sources, most notably the BMBL (**http://www.cdc.gov/od/OHS/biosfty/bmbl5/bmbl5toc.htm**). However, low risk human pathogens that are designated as BSL2 agents under normal laboratory procedures and practices may be classified as BSL2 with enhanced precautions because of the potential for aerosol and/or splash exposure associated with cell sorting; thus cell sorting is considered a laboratory procedure hazard. Therefore, an aerosol management system is required at all biosafety levels and usually consists of an evacuation pump equipped with a HEPA filter. All aerosol management systems require validation (as shown below), although the frequency of testing increases with increased biosafety levels. General Guidelines for the determination of biosafety levels and procedures for cell sorting are shown in Table 1 below. Specific examples of agents and their biosafety level for cell sorting are shown in Appendix I.

Table 1: Biosafety Level Determination for Cell Sorting

	BSL2	BSL-2 with enhanced precautions (during sorting operations)	BSL3	BSL4
Risk Assessment Condition	**Uninfected non-primate**	**Non-infectious Human /NHP cells** **Infectious but with low risk assessment**	**Infectious samples with high risk assessment** **All samples containing known aerosol pathogens**	**Extremely Dangerous Pathogens**
Example Sample type or Agents[1]	Normal murine cells 3rd gen Lentivirus (non-human cells)	Normal human blood Human cell lines[1] An example agent is: Influenza A[1] 2nd gen Lentivirus or 3rd gen in human cells	Example agents include[1]: Mycobacterium Tuberculosis, Monkeypox	Example agents include[1]: Ebola, Marburg
Containment System Validated	Periodically (monthly or with filter change)	Periodically (monthly or with filter change)	Before Every Sort	Before Every Sort
Aerosol Containment Operational	Required	Required	Required	Required
N-95, N-99 or N-100 respirator	Optional	Required[2]	N/A	N/A

PAPR	Optional	Optional	Required	N/A
Eye protection	Safety Glasses	Face shield or safety goggles	N/A	N/A
Lab Coat	Front Closure lab coat	Wrap around rear closure	Coveralls	Special suit
Separate Room and Environmental controls	Optional	Required or limited access to room[3]	Required[4]	Required[4]

[1]Example Sample type or Agents - the samples and/or agents listed represent only a partial list of agents which may be included in each category. A risk assessment should be conducted for all samples/agents prior to sorting, and the appropriate biosafety level determined in collaboration with safety specialists, subject matter experts and the NIH IBC. For additional information please consult the following web sites: http://www.phac-aspc.gc.ca/msds-ftss/index-eng.php; http://www.cdc.gov/od/OHS/biosfty/bmbl5/bmbl5toc.htm

[2]. Respirators must remain on during all procedures associated with sample manipulation, including sample tube cap removal and loading of sample on instrument, or when removing collection tubes or other procedures where the sort or collection chamber is opened. Note that respirator protection may otherwise be removed during the sorting process providing the aerosol management system is active and all sort chamber and collection chamber doors are closed. For human pathogens that are classified as BSL2 and are not respiratory hazards, but which may pose a risk if exposed to mucous membranes, only mucous membrane protection is required. Examples of agents in this category include Leishmania and toxoplasmosis in murine cells

[3]Enclosure of the cell sorter within a Class II BSC (NSF-49 certified) may abrogate the need to house the sorter in a separate room within the BSL2 lab space; PPE (as detailed above) is optional, but strongly encouraged for the operator during procedures requiring manipulation of instrument. Cell sorters located within a shared laboratory may be operated under BSL2 with enhanced precautions if during the operation of the sorter, access to the room is limited and PPE as detailed above is worn by all occupants

[4]Enclosure of cell sorter within a Class II (NSF-49 certified) BSC required

Standard Operating Procedure Development

An important outcome of any risk assessment process is the creation of SOP's. The SOP takes into account hazards (agent and laboratory procedure) and specifies practices and procedures designed to minimize or eliminate exposures to those hazards. For cell sorters, the design of the instrument, especially containment or aerosol evacuation components, must be considered in the development of the SOP. Each instrument must be evaluated for deficiencies in containment or aerosol evacuation design and appropriate procedures adopted to minimize risk. An important example of this is that most cell sorters do not possess an interlock designed to prevent the operator from opening the sort chamber after a nozzle obstruction with subsequent stream deviation. Therefore, the SOP should clearly address the procedures for evacuating the sort chamber of aerosols prior to opening the sort chamber, including a stated time period to wait after a clog induced stream deviation.

The general considerations for SOP development are outlined below:

1. Preparation before the sort
    a. Check fluids, empty waste
    b. Cover control surfaces with plastic wrap, including keyboards and mouse.
    c. Perform containment testing
    d. Verify any automated decontamination functions
    e. Preparation of disinfectant solutions
    f. Sample preparation, i.e. staining, centrifugation, pipetting or manipulations that may generate aerosols should be performed in a manner to maximize containment and protect the worker
2. Procedures in the event of a nozzle obstruction
    a. Turn off stream
    b. Evacuate sort chamber prior to opening; increase Aerosol Management System (AMS) evacuation rate
    c. Attempt to clear nozzle clog by stream flush routines, with sort chamber door closed. If clog is not cleared, remove the nozzle and dependent upon sample risk assessment, decontaminate nozzle before sonication
3. Decontamination procedures
    a. Decontaminate and clean sample lines, sort chamber and collection chamber
    b. Decontaminate and clean surfaces around cytometer, especially near the sort chamber

Development of the SOP should also include consultation with NIH safety specialists who can provide guidance on general biosafety procedures as well as information on NIH policy. Examples of SOP's for cell sorters are included in Appendix II to serve as templates for development of individual laboratory SOP's. Finally, the SOP should be reevaluated at least on an annual basis or whenever there is a change in instrument configuration that may affect biosafety.

NIH Biosafety Policy for Cell Sorters

Room design (Adapted and extended from BMBL, 5th Ed., Section IV)

1. **Biosafety Level 2 laboratory** -General
   1.1. Air flow in the room is balanced to create negative airflow into the room. It is recommended that a visual monitoring device be located at the door to measure negative airflow.
   1.2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
   1.3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
   1.4. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
   1.5. An eyewash station must be readily available.
2. **Biosafety Level 2 with enhanced precautions**
   2.1. Ideally, the cell sorter is located in a separate, lockable room where no other laboratory activity is performed. If the sorter is located in shared laboratory space, all PPE requirements (as outlined below and in Table 1) should be followed by all personnel during sorting procedures. The cell sorter should be placed in a location in the lab so that directional air flow is toward the cell sorter and away from other areas of the lab. If the cell sorter is enclosed within a Class II BSC (NSF-49 certified), the requirement for placement of the cell sorter in a separate room may be abrogated dependent upon the overall risk assessment.
   2.2. Air flow in the room is balanced to create negative airflow into the room. It is recommended that a visual monitoring device be located at the door to measure negative airflow.
   2.3. The sorting room is locked to restrict access and allow the operator to concentrate on the sort and to maintain regular air flow and negative air pressure in the room.
   2.4. During sorting procedures, a sign should be placed on the outside of the door to indicate that a potentially biohazardous sort is in progress. This sign should also contain all necessary information for entering the room safely, including warning for Class IIIb or IV lasers if applicable.
3. **Biosafety Level 3 Laboratory**
   3.1. The cell sorter must be located within a Class II BSC (NSF 49 certified; can be recirculated).

Cell Sorter Specific Safety Equipment and Practices

4. **Aerosol Containment**
   4.1. Aerosol management System (AMS): All cell sorters must be equipped with an aerosol management or evacuation system which is designed to evacuate the sort chamber and sort collection area of the cytometer. It consists of an evacuator that creates negative pressure within those chambers and transports aerosols through a HEPA or ULPA filter before exhausting to the room. The AMS should be operated under all biosafety levels, BSL2 and BSL2 with enhanced precautions, BSL3 and BSL4.
      4.1.1. Validation of Aerosol containment systems
         4.1.1.1. Currently, the most widely accepted method of containment testing utilizes fluorescent plastic beads which are run on the instrument as a sample (2, 3). The AMS must be tested under simulated worse case "failure mode". In this mode the instrument is set to high pressure (usually 70psi) and fluorescent particles are concentrated to approach speeds of approx. 20,000-50,000 particles/second. The stream is forced to glance off of the waste catcher shield to create a large plume of aerosols and aerosols concentrated on a slide for subsequent analysis on a microscope. Tolerance of aerosol escape is zero particles when the AMS is active and sort chamber door is closed. This test is performed periodically (monthly or only when filters are exchanged) for BSL2 labs and for labs performing sorts under BSL2 with enhanced precautions and performed prior to every sort for BSL3 labs.
         4.1.1.2. Details of this procedure are in Appendix III.

User specific safety equipment

5. **Personal Protective Equipment (PPE) for Biosafety Level 2 Laboratory**
   5.1. Front closure lab coat and gloves
   5.2. Eye Protection: Safety glasses
6. **Personal Protective Equipment (PPE) for Biosafety Level 2 with enhanced precautions:**
   6.1. Isolation style solid-front or wrap around gown and gloves
   6.2. **Eye protection:** Safety goggles, face shield, splatter guard or integral respirator/face shield which provide mucous membrane protection as required for anticipated splashes or sprays of infectious or other hazardous materials.
   6.3. Respirator:
      6.3.1. NIOSH approved respirators must be worn during operation of the cell sorter at this biosafety level. Approved respirators include N-95, N-99 or N-100 filtering facepiece respirators or powered air purifying respirators (PAPR) with integral face shield. For non-primate samples containing agents that do not pose respiratory risk, mucous membrane protection may be substituted for respirators. For example, the

human pathogens leishmania and mouse models of toxoplasma infection are included in this category.

6.3.2. All individuals using respirators must be enrolled in the NIH Respiratory Protection Program. Supervisors are not authorized to select respiratory protective devices. If you have any questions, contact the DOHS Technical Assistance Branch at 301-496-3353

7. Personal Protective Equipment (PPE) for Biosafety Level 3 Laboratory:

7.1. Liquid-resistant scrub suits or coveralls gloves (double pair). Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Gloves and protective clothing must not be worn outside the laboratory and must be disposed of with other contaminated waste.

7.2. Eye Protection/Respirator:

7.2.1. NIOSH-approved powered air purifying respirators (PAPR) with integral face shield must be worn at all times when in the laboratory. If you have any questions, contact the DOHS Technical Assistance Branch at 301-496-3353. Eye and face protection must be disposed of with other contaminated waste or decontaminated before reuse.

Disinfection:

8. The choice of disinfectant is dependent upon a variety of factors (4) including the agent in use, the chemical resistance of the cell sorter components and potential of exposure of lab personnel to the chemical disinfectant. Broad spectrum disinfectants are desirable in a facility in which agent use is varied. Sodium hypochlorite solutions (1:10 dilution of household bleach in H_20; 5,250-6,150 ppm of chlorine) offer several advantages over alcohols and other disinfectants; bleach has broad spectrum antimicrobial activity, does not leave toxic residues, is unaffected by water hardness and is inexpensive and fast acting. However, because of the corrosive nature to metals, exposure to instrumentation should be limited to times determined to be maximally efficacious to microbial killing. In addition, bleach solutions must be prepared fresh due to loss of free available chlorine. However, there are commercially available sprayers that mix the bleach and water when sprayed, eliminating the need to make fresh solutions daily.

Appendix I: Example Agent List with Biosafety Level for Cell Sorting[1]

Agent	Recommended Biosafety Level	Restrictions or Comments	MSDS Link
Hepatitis C	BSL2+[2]		http://www.phac-aspc.gc.ca/msds-ftss/msds77e-eng.php
Human Metapneumovirus	BSL2+		
Human Parainfluenza Virus type 3	BSL2+		
Influenza A	BSL2+	Influenza (seasonal) vaccine required	
Klebsiella pneumonia	BSL2+		http://www.phac-aspc.gc.ca/msds-ftss/msds90e-eng.php
LaCrosse virus	BSL2+		
LCMV	BSL2+ or BSL3	Ensure that HVAC system does not exhaust near vivarium housing mice; BSL dependent upon strain; pregnant women should consult Occupational Medical Service (OMS) or their personal physician prior to performing a procedure with this agent.	http://www.phac-aspc.gc.ca/msds-ftss/msds97e-eng.php
Leishmania	BSL2+[3]		http://www.phac-aspc.gc.ca/msds-ftss/msds94e-eng.php
Malaria	BSL2+[3]		
PVM (Pneumonia Virus of Mice)	BSL2+		
Respiratory Syncytial Virus	BSL2+		http://www.phac-aspc.gc.ca/msds-ftss/msds125e-eng.php
Toxoplasma gondii	BSL2+	Pregnant women should consult OMS or their personal physician prior to performing a procedure with this agent.	http://www.phac-aspc.gc.ca/msds-ftss/msds153e-eng.php
Vaccinia	BSL2+	vaccine required	http://www.phac-aspc.gc.ca/msds-ftss/msds160e-eng.php
1918 Influenza	BSL3	Influenza (seasonal) vaccine required	
Avian influenza	BSL3	Influenza (seasonal) vaccine required	
H1N1	BSL3	H1N1 vaccine required;	
HIV	BSL2+ or BSL3		http://www.phac-aspc.gc.ca/msds-ftss/msds84e-eng.php
Monkeypox	BSL3	vaccinia vaccine required, every 3 years	
TB, Mycobacterium tuberculosis	BSL3		http://www.phac-aspc.gc.ca/msds-ftss/msds103e-eng.php

[1]This list represents examples of biosafety level determination for cell sorting of specific agents. The final determination of the biosafety level is dependent upon the risk assessment conducted in collaboration with safety specialists, subject matter experts and the NIH IBC

[2]BSL2 with enhanced precautions is abbreviated BSL2+ for this table

[3]Respirator PPE optional (mucous membrane protection required) for this agent except where the sample also contains human/NHP blood cells or fluids.

Appendix II: Standard Operating Procedure examples

These can be used as guidelines for formulating a Standard Operating Procedure for individual laboratories. Procedures and practices will vary dependent upon risk assessment and instrument designs. Product or company names used in these examples do not in any way constitute explicit or implicit endorsement of these products or companies by the NIH.

BSL2 SOP

1. Wear Lab Coat and Gloves

2. Turn on Aerosol Management System (biohazard vacuum) and operate at 20% or as recommended by instrument manufacturer

    a. Check vacuum reading. If vacuum is >2.4 inches of H_2O, change HEPA filter. Note: HEPA filter must be changed every 6 months, regardless of vacuum reading.
    b. Procedure for changing HEPA filter on AMS unit:
        i. While wearing gloves, lab coat, N-95 rated face mask (respirator) or PAPR and goggles/safety glasses, place the Buffalo unit HEPA filter inside an orange biohazard plastic bag. Disconnect hose from the Aria and also place within the bag. Seal the bag and place within a Medical Pathological Waste (MPW) box. Install a new HEPA filter and hose.

3. Make sure collection chamber door and sort chamber door are closed during sorting procedures

4. Do not eat or drink in laboratory

5. Remove gloves before answering phone

6. Remove lab coat and gloves and wash hands before leaving lab

BSL2 with enhanced precautions SOP - FACS Aria II

1. **<u>Preparation before the sort</u>**

    a. Cover keyboard, mouse and other instrument control surfaces w/ plastic wrap; clear surfaces of clutter, use absorbent pads for samples.
    b. Using a damp paper towel(s), wipe up dried bleach residue from instrument areas, paying particular attention to the sample uptake area, O-rings, charge plates and the side stream viewing window. Warning: Failure to remove salt residue from the sample uptake system may cause the pressurized seal to fail and release potential aerosols!
    c. Prepare sort collection chamber as necessary. Install the correct collection tube holder. Close sort collection chamber door.
    d. Turn biohazard vacuum (Buffalo Filter Whisper Unit) on and operate at 20%. Check vacuum reading. If vacuum is >2.4 inches of H_2O, change HEPA filter. Note: HEPA filter must be changed every 6 months, regardless of vacuum reading.
        i. Procedure for changing HEPA filter on AMS unit:
            1. While wearing gloves, lab coat, N-95 rated face mask (respirator) or PAPR and goggles/safety glasses, place the Buffalo unit HEPA filter inside an orange biohazard plastic bag. Disconnect hose from the Aria and also place within the bag. Seal the bag and place within a Medical Pathological Waste (MPW) box. Install a new HEPA filter and hose.
    e. Make sure sheath tank is filled and standard waste tank contains enough bleach to give a final 10% (1:10 dilution of household bleach) solution when filled. Fill a spray bottle with a freshly made 10% (1:10 dilution) bleach solution for work area decontamination.
    f. Wear gloves, lab coat, N-95 rated face mask (respirator) or PAPR and goggles/safety glasses (or N-95 mask with face shield) before handling samples. Lab door must be closed.
    g. Respirators <u>must</u> remain on during all procedures associated with sample manipulation, including sample tube cap removal and loading of sample on instrument, or when removing collection tubes or other procedures where the sort or collection chamber is opened as outlined below in Sections 3. Note that respirator protection may otherwise be removed during the sorting process except during procedures as outlined above.
    h. Have a spare nozzle, with new O-ring installed, available in case of a clog.
2. **<u>Procedures during sorting/analysis</u>**
    a. Filter samples prior to sort to avoid clogs
    b. Fill sample tube with as much sample as possible to minimize loading and unloading sample. DO NOT fill higher than ¼ inch from the top of the tube.
    c. Make sure the "Sweet Spot" is enabled.
    d. Close sort collection chamber door before starting sample.
    e. When changing collection tubes:

f. Stop the sample flow and close the aspirator drawer by clicking the Acquire button.
g. Wait at least 60 seconds before opening sort collection chamber door.
h. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, use alcohol swab or bleach to wipe outside of tubes.

3. **Procedures in the event of a nozzle obstruction**

   a. If during the sort the stream is deflected (due in part to a clogged nozzle), the sort is designed to stop automatically and block the sort tubes. The sort will not restart until the operator has cleared the clog. In the event of a nozzle clog , DO NOT open sort collection chamber door or sort block door before following this procedure:
      i. If the system has not already shut down automatically, turn off the stream using the button labeled with an '✓' on the Breakoff window. This will shut off the stream, unload the sample and close the aspirator door.
      ii. Open aspirator drawer using software controls.
      iii. Increase the air evacuation rate on the AMS unit to 100%.
      iv. Wait at least 60 seconds. This procedure will clear aerosols from the sort chamber. . (Note that this step assumes that a modification to tube holder(s) (universal top component on Aria II) involving the drilling of 3 holes and the sort chamber door involving the drilling of 1 hole with attachment of 0.22μm filter, has been previously performed. (1))
      v. Close the aspirator drawer.
      vi. With the sort block chamber door, aspirator drawer and collection chamber door all closed, turn the stream on and off several times or perform the 'Clean flow Cell' procedure with DI H20 followed by turning the stream on to see if the clog will clear itself.
      vii. Turn stream off.
      viii. Open the aspirator drawer and evacuate for at least 60 seconds before closing the aspirator drawer again
      ix. The sort block chamber door and sort collection chamber door can now be opened.
      x. If it is necessary to change nozzles, remove nozzle and O-ring and place in tube with 10% (1:10 dilution) bleach for 30 minutes. Thoroughly rinse nozzle in water and let air-dry. Discard O-ring if not using nozzles with integrated O-rings. Spare integrated nozzle or spare nozzle with O-ring may be installed while obstructed nozzle is soaking in bleach.
      xi. With stream turned off, open the sort block chamber door and dry plates and surfaces as needed.
      xii. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, use alcohol swab or bleach to wipe outside of tubes.
      xiii. Set AMS unit to 20% vacuum.
      xiv. Make sure that all chamber doors are closed and restart the stream.

4. **Decontamination Procedures:**
   a. Disengage "Sweet Spot" and turn the stream off.
   b. Disinfect sample lines using a freshly made 10% bleach solution as follows:

i. Fill a tube with a volume of 10% bleach equal to or greater than the volume of sample that was sorted and place on the sample stage.
ii. Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell. Perform this step three times or until a bleach drop is visible in the stream camera view.
iii. Wait 30 or more minutes with 10% bleach in flow cell.
iv. Fill a tube with DI water, Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell
v. Fill a tube with 70% ETOH, Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell. Perform this step three times or until an ETOH drop is visible in the stream camera view. Shutdown instrument.

c. Clean all surfaces around optical bench, sort block chamber and charge plates, sort collection chamber, sample introduction area and sample tube holder(s) with a prepackaged 10% bleach towel and/or 10% (1:10 dilution) bleach from a spray bottle. Clean keyboard cover, remove any plastic wrap and discard in MPW box.
d. When leaving the lab:
   i. Make sure all samples are capped.
   ii. Remove gloves, respirator & lab coat (remember outside of gloves are contaminated!).
   iii. WASH HANDS!

BSL3 SOP - FACS Aria II

1. **FACS Aria Cell Sorter**
    a. The FACS Aria Cell Sorter and associated Aerosol Management System are located within a Class II Biological Safety Cabinet with Dual door assembly. The hood doors must be closed with sash windows in the closed position and the hood operational during all procedures involving infectious agents.
    b. Instrument Pre-Sort Check and Supplies Check must be performed as outlined in Section 3 below.

2. **Aerosol Management System (AMS)**

    a. While sorting viable infectious material (infected cells) the following guidelines must be followed for proper aerosol containment. All sort operators in this section must be trained and certified by the Flow Cytometry Section prior to any cell sorting operations.
    b. The AMS must be on and functioning according to the manufacturer guidelines. The vacuum control should be set to 20% and the vacuum gauge less than 2.4 inches of H_2O. If it is outside of this range, replace HEPA filter unit and tubing.
    c. HEPA filter must be changed under the following conditions:
        i. The vacuum monitor gauge reads 2.4 inches of H_2O or greater at 20% suction.
        ii. The red filter indicator LED is blinking.
        iii. Three months has passed since installation of the filter.
    d. Care must be taken when removing filter and associated hose since these are assumed contaminated. After changing the hose and filter, ensure that the Filter Life Reset button has been pushed and the new filter has been dated.
    e. 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 Sweetspot which is used during all sorting operations. This device can detect stream drifts and possible clogs and automatically shutdown the stream.

3. **Preparation before the sort: Instrument Pre-Sort Check and Supplies Check**
    a. Bleed Fluidics Cart filters
    b. Fill sheath tank. Empty waste if necessary by thoroughly mixing closed waste container, and disposing in sink followed by large amounts of water. Fill waste tank with 10% final bleach concentration. Prepare fresh 10% bleach solution daily.
    c. Close BSC cabinet doors.
    d. Turn on Instrument and launch software.
    e. Using a damp paper towel(s), wipe up dried bleach residue from instrument areas, paying particular attention to the sample uptake area, O-rings, charge plates and

the side stream viewing window. Warning: Failure to remove salt residue from the sample uptake system may cause the pressurized seal to fail and release potential aerosols.

f. Open sort block chamber door and verify that aspirator door is operational using software controls
g. Perform instrument Quality Control procedures.
h. Verify that all supplies are stocked.

4. Procedures during infectious sort

a. The flow cytometer must pass all tolerances of aerosol containment as described in Appendix III. If these tolerances are not met, infectious cell sorting is not permitted.
b. The Class II Biological Safety Cabinet must be turned on.
c. Turn on and verify that the AMS is working correctly. This device must have a vacuum pressure of <2.4 inches of H_2O. If this tolerance is not met, infectious cell sorting is not permitted.
d. Close all barriers around the sort chamber. If this is not done, infectious cell sorting is not permitted.
e. While within a Class II Biosafety cabinet, all samples for sorting must be filtered through a 50 µm mesh prior to sorting. This reduces the potential for clogging and decreases the risk of creating aerosols.
f. Place sample onto the sample station. Start sort and monitor the sort performance using the Accudrop camera.

5. Procedures in the event of a nozzle obstruction

a. If during the sort the stream is deflected (due in part to a clogged nozzle), the sort is designed to stop automatically and block the sort tubes. The sort will not restart until the operator has cleared the clog. In the event of a nozzle clog , DO NOT open sort collection chamber door or sort block door before following this procedure:
    i. If the system has not already shut down automatically, turn off the stream using the button labeled with an '✓' on the Breakoff window. This will shut off the stream, unload the sample and close the aspirator door.
    ii. Open aspirator drawer using software controls.
    iii. Increase the air evacuation rate on the AMS unit to 100%.
    iv. Wait at least 60 seconds. This procedure will clear aerosols from the sort chamber. (Note that this step assumes that a modification to tube holder(s) (universal top component on Aria II) involving the drilling of 3 holes and the sort chamber door involving the drilling of 1 hole with attachment of 0.22µm filter, has been previously performed. (1))
    v. Close the aspirator drawer.
    vi. With the sort block chamber door, aspirator drawer and collection chamber door all closed, turn the stream on and off several times or perform the 'Clean flow Cell' procedure with DI H20 followed by turning the stream on to see if the clog will clear itself.
    vii. Turn stream off.

viii. Open the aspirator drawer and evacuate for at least 60 seconds before closing the aspirator drawer again
ix. The sort block chamber door and sort collection chamber door can now be opened.
x. If it is necessary to change nozzles, remove nozzle and O-ring and place in tube with 10% (1:10 dilution) bleach for 30 minutes. Thoroughly rinse nozzle in water and let air-dry. Discard O-ring if not using nozzles with integrated O-rings. Spare integrated nozzle or spare nozzle with O-ring may be installed while obstructed nozzle is soaking in bleach.
xi. With stream turned off, open the sort block chamber door and dry plates and surfaces as needed.
xii. When removing collection tubes, be aware that the outside of the tube is potentially contaminated, use alcohol swab or bleach to wipe outside of tubes.
xiii. Set AMS unit to 20% vacuum.
xiv. Make sure that all chamber doors are closed and restart the stream.

6. <u>Decontamination Procedures:</u>

a. Disengage "Sweet Spot" and turn the stream off.
b. Disinfect sample lines using a freshly made 10% bleach solution as follows:
   i. Fill a tube with a volume of 10% bleach equal to or greater than the volume of sample that was sorted and place on the sample stage.
   ii. Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell. Perform this step three times or until a bleach drop is visible in the stream camera view.
   iii. Wait 30 or more minutes with 10% bleach in flow cell.
   iv. Fill a tube with DI water, Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell
   v. Fill a tube with 70% ETOH, Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell. Perform this step three times or until an ETOH drop is visible in the stream camera view.
   vi. Replace integrated nozzle with closed loop nozzle.
   vii. Perform Instrument Shutdown as prompted in software; use 70% EtOH for cleaning solution. Place sample tube holder into tube containing 10% bleach for 30 minutes. Wash tube holder and cam in DI water and let air dry.
   viii. Remove sheath probe and drain residual sheath from probe. Place probe on absorbent paper in hood. Empty sheath tank and place in bag for autoclaving.
   ix. Turn off AMS. Verify that vacuum gauge is at zero.
   x. Clean all surfaces around optical bench, sort block chamber and charge plates, sort collection chamber, sample introduction area and sample tube holder(s) with a prepackaged 10% bleach towel and/or 10% bleach from a spray bottle.

Appendix III: Procedure for Validation of aerosol containment

1. Measurement of Containment and Tolerances

    a. Assemble multiple-jet single-stage impactor (AeroTech 6), flow meter and vacuum trap as shown in Appendix IV.

    b. Turn AMS on (20%) and check for proper vacuum function (<2.4 inches of H_2O).

    c. The AMS must be tested under simulated worse case failure mode. In this mode the instrument is set to 70 psi with the stream glancing off of waste-trough to create large aerosols. This is done by first adjusting the retaining screws on either side of the sort block chamber (see appendix V) followed by moving the chamber until stream deflection is observed on the accudrop monitor.

    d. Close the Sort Block Chamber door. Add a glass slide (in a 100mm Petri dish) to the AeroTech impactor and place directly on top of collection chamber (see appendix VI). Adjust the vacuum pressure to the AeroTech impactor to 35 LPM (liters per minute). Open the Aspirator Door using the software control but do not install tube holders.

    e. Place Glo-Germ particles onto the sample station and adjust either the particle concentration or the flow rate to achieve a particle rate of at least 50,000 particles per second (Appendix VII).

    f. Begin acquiring Glo-Germ particles and allow collection for 5 minutes (Sample 1). The Bio-Protect II Class II safety cabinet that houses the instrument should NOT be turned on in order to more accurately assess containment of the AMS.

    g. Turn off vacuum to AeroTech impactor and remove slide from inside. Put in a fresh slide and locate the AeroTech impactor in front of the collection chamber (Appendix VI). Turn on the vacuum, adjust to 35 LPM and collect for 5 minutes (Sample 2).

    h. Turn off vacuum to AeroTech impactor and remove slide from inside. Put in a fresh slide and locate the AeroTech impactor under the sample station (Appendix VI). Turn on the vacuum, adjust to 35 LPM and collect for 5 minutes (Sample 2) and collect for 5 minutes (Sample 3).

    i. Turn off vacuum to AeroTech impactor and remove slide from inside. Put in a fresh slide and locate the AeroTech impactor on top of collection chamber and open the Sort Block Chamber Door partially (Appendix VI). Turn AMS off,

close the aspirator door using the software control. Turn on the vacuum, adjust to 35 LPM and collect for 5 minutes (Sample 4). This is the Positive Control. Turn off vacuum, close front cover and verify that particles are still at or above the 50,000/sec flow rate.

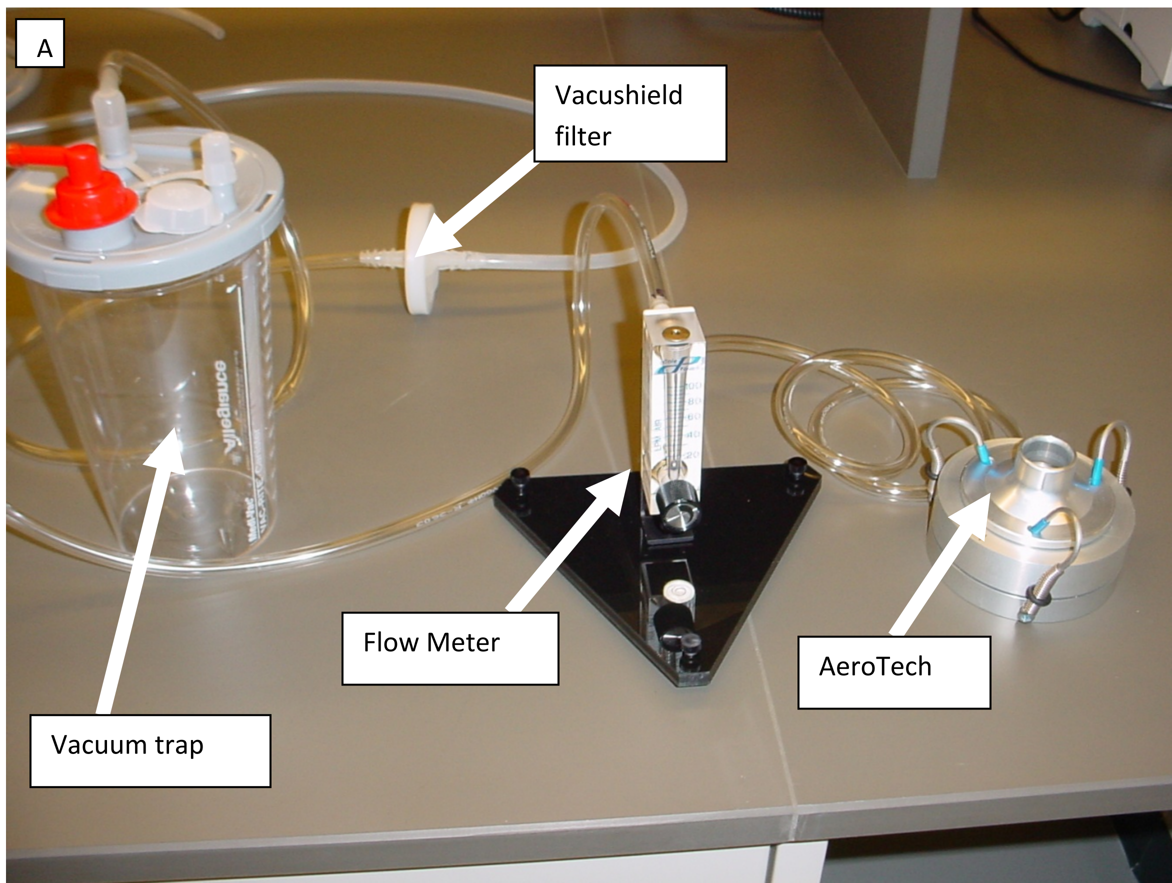
j. Examine glass slides for bright green fluorescence using a fluorescent microscope equipped with a FITC filter (520-640nm). See Appendix VII.

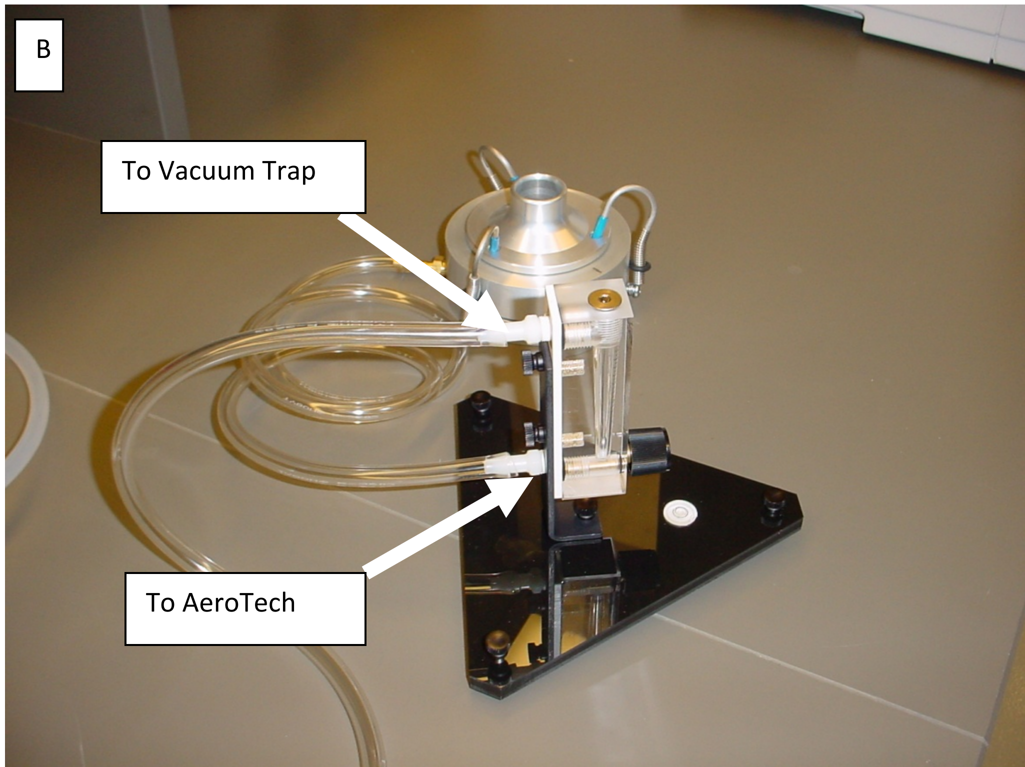
k. Scan the entire slide on 10X and count all Glo-Germ particles. The positive control slide can be used as a reference if the slide reader needs help to distinguish between fluorescent debris and actual Glo-Germ particles. Record all data, see appendix VIII.

l. Acceptable Tolerance: No Glo-Germ particles detected after 5 minutes of active air sampling in Samples 1, 2 and 3. The positive control slide (sample 4) must contain greater than 100 particles after 5 minutes of active air sampling with the AMS turned off and no tube holder in place.

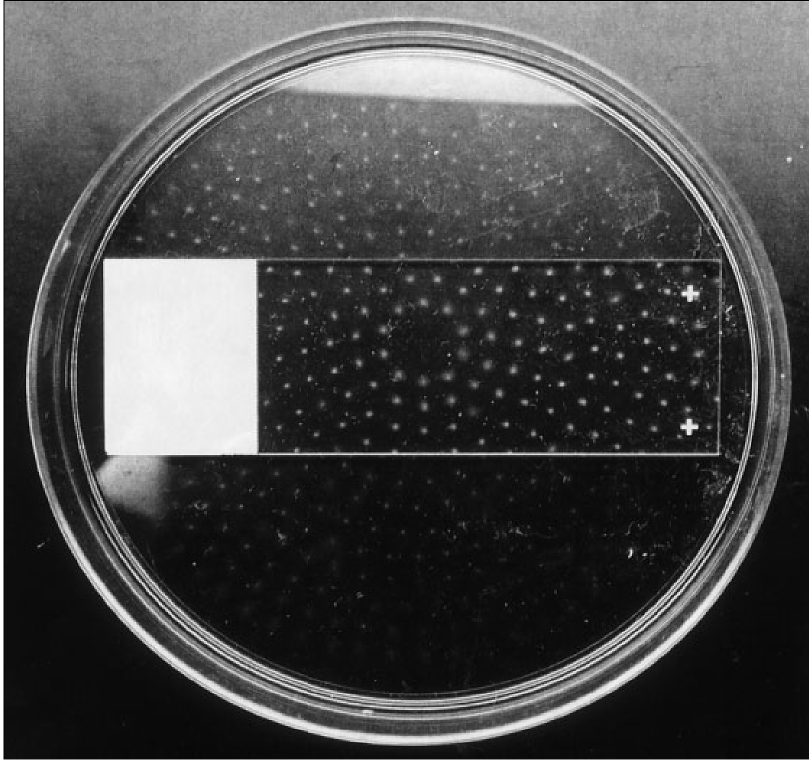
Appendix IV: Aerotech collection system setup

A. Picture showing Aerotech Setup consisting of Aerotech 6 impactor, flowmeter and vacuum trap. (AeroTech 6TM viable microbial particle sampler, Cat. No. 6TM (AeroTech Laboratories, Inc., Phoenix, AZ) (www.aerotechlabs.com)
B. Flow Meter connections (Cole Parmer Flow Meter: Catalog Number EW-32460-52 (meter). Catalog number EG-32462-50 (Stand).
C. Disassembled Aerotech device showing slide placed inside Petri dish
D. Glo-Germ particles deposited on a slide placed within sort chamber (positive control). (Glo Germ™ Particles (GLo Germ Inc., Cat. No. GGP, (www.glogerm.com))



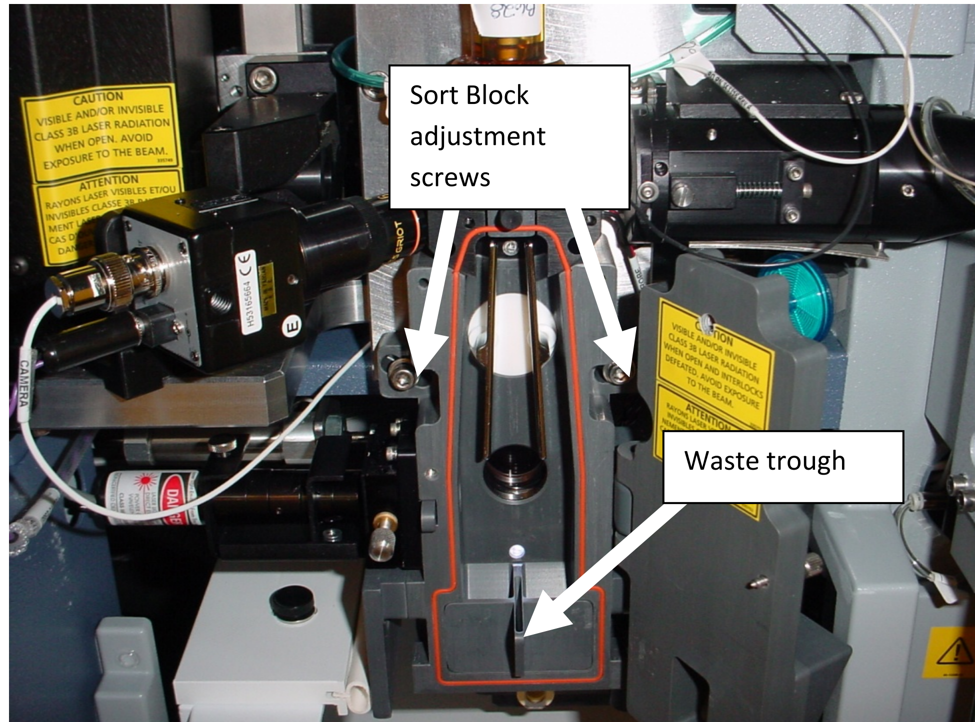

B
To Vacuum Trap
To AeroTech

C



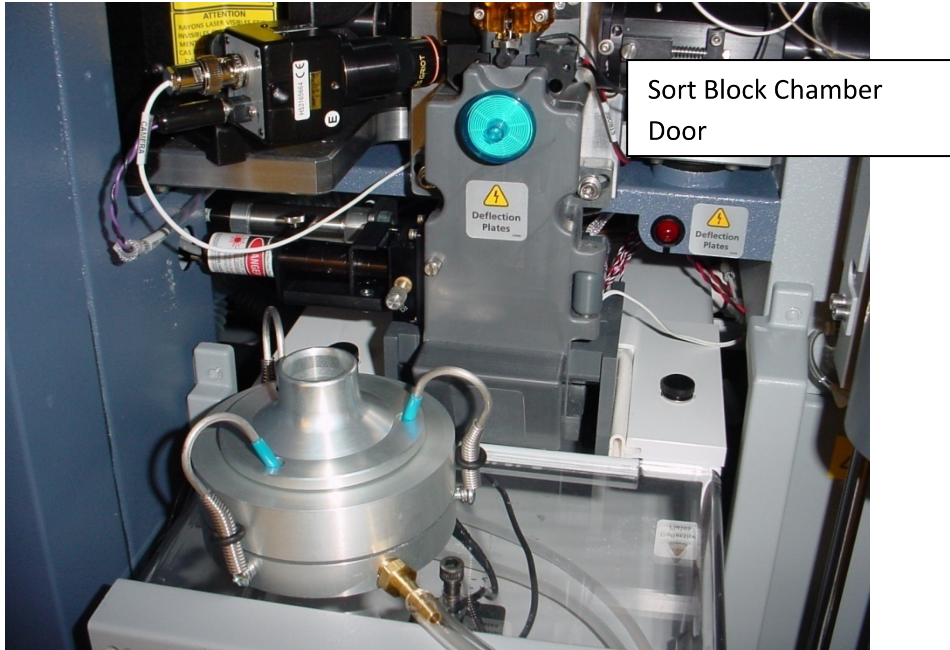
Appendix V: Sort block chamber adjustment for AMS tests

To create a ‘failure mode’ condition, in order to perform the Glo-Germ AMS testing procedure, adjust hex screws as shown in order to direct center stream to hit the edge of the waste trough.



Appendix VI: Aerotech placement for GloGerm Testing

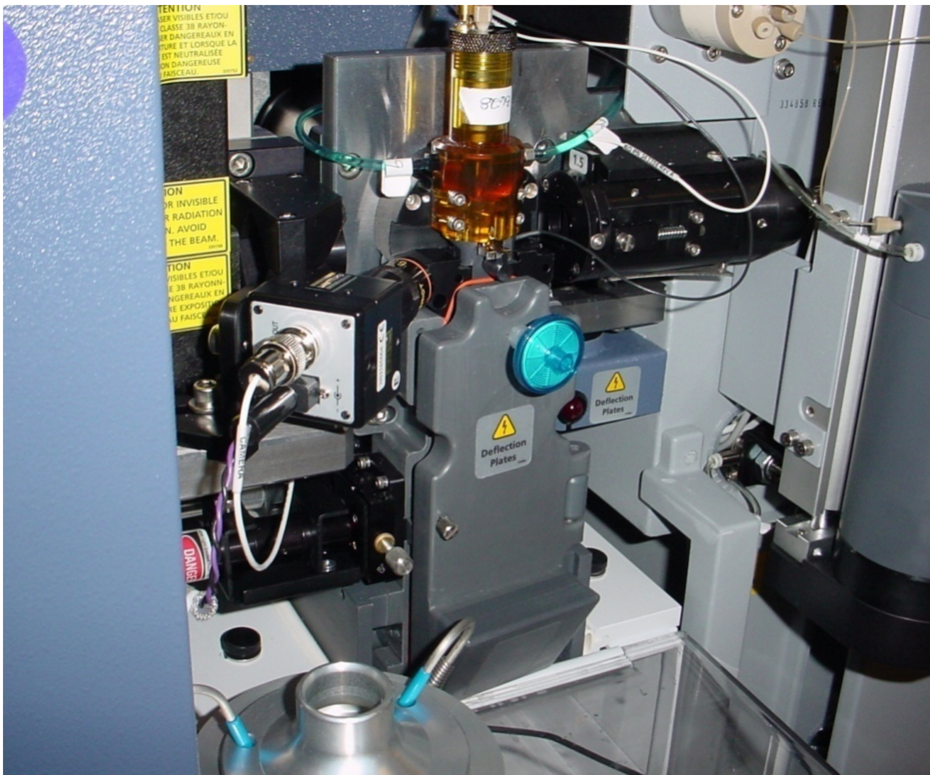
SAMPLE 1: TOP OF COLLECTION CHAMBER



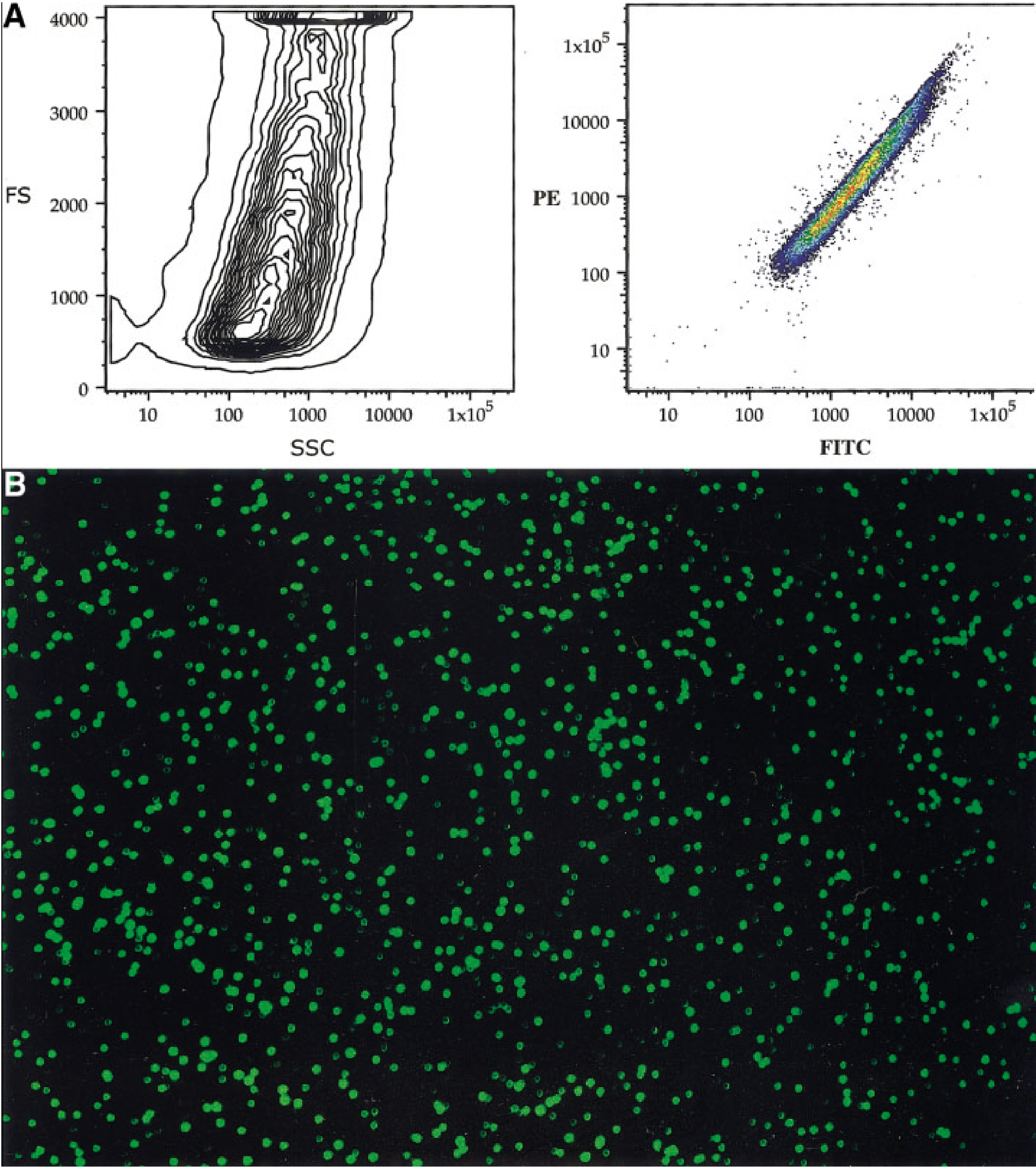
SAMPLE 2: IN FRONT OF COLLECTION CHAMBER

SAMPLE 3: UNDER SAMPLE STATION

SAMPLE 4: ON TOP OF COLLECTION CHAMBER, DOOR PARTIALLY OPEN



Appendix VII: Histogram and microscope views of GloGerm particles



Appendix VIII: Example Containment test record sheet

DATE:	
OPERATOR:	

RESULT:

TEST SAMPLES:

SAMPLE 1: [ ]

AMS ON
SBC DOOR CLOSED
ASP DOOR OPEN
POSITION: TOP OF COLLECTION CHAMBER

SAMPLE 2: [ ]

AMS ON
SBC DOOR CLOSED
ASP DOOR OPEN
POSITION: FRONT OF COLLECTION CHAMBER

SAMPLE 3: [ ]

AMS ON
SBC DOOR CLOSED
ASP DOOR OPEN
POSITION: UNDER SAMPLE STATION

POSITIVE CONTROL:

SAMPLE 4: [ ]

AMS OFF
SBC DOOR PARTIALLY CLOSED
ASP DOOR CLOSED
POSITION: TOP OF COLLECTION CHAMBER
(TO THE RIGHT OF SC)
COMMENTS:

AEROTECH ON,
BIOPROTECT II HOOD OFF,
STREAM FAILURE MODE
FOR ALL TESTS;

Abbreviations:

SBC	Sort Block Chamber
ASP	Aspirator
AMS	Aerosol Management System

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2. Oberyszyn AS. Method for visualizing aerosol contamination in flow sorters. Curr Protoc Cytom 2002;Chapter 3:Unit 3.5.

3. Perfetto SP, Ambrozak DR, Koup RA, Roederer M. Measuring containment of viable infectious cell sorting in high-velocity cell sorters. Cytometry A 2003;52:122-130.

4. Rutala WA, Weber DJ, HICPAC. Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008. 2011.