Purpose: To define Flow Cytometry Core Facility procedures for sorting with BSL2 with enhanced precautions

Scope: This procedure is to be utilized for all Health Science Center (HSC) Flow Cytometry Core Facility laboratory safety practices and procedures at the University of Utah.

Contents:

Cross Referenced Documents:

SOP 5 Wintrobe Aria Usage Instructions
SOP 6 HCI Aria Usage Instructions
SOP 33 Cyclex-D Aerosol Containment Testing
SOP 42 Spill and Exposure Plan
Aerosol Containment Results Log
Risk Assessment Form

Terms used in this document or other related safety documents:

IBC = Institutional Biosafety Committee
BSC = Biosafety Cabinets (defined as Class I, II or III)
BSL = Biosafety Level (defined as 1, 2, 3 or 4)
BSL2+: BSL2 with enhanced precautions or BSL2 containment with BSL3 practices
HEPA = High Efficiency Particle Absorber
PPE = Personal Protective Equipment

1.0 Introduction

The Flow Cytometry Core Facility has two BD FACSAria cell sorter locations. Proximity card and magnetic locking systems restrict access to Room 221 in Wintrobe. Building access is restricted to HCI RN. UID card access is accomplished through the HSC Cores administration office or HCI administration.

Each Aria is encased within a bioBUBBLE Class I Benchtop Biocontainment Enclosure (BBE). These BBEs serve as containment solutions for cell sorters because they capture aerosols and other airborne particulates generated during cell sorting. BBEs protect the operator and lab space by containing biological hazards, allowing Risk Group II materials to be sorted in compliance with BSL-2 and BSL-2+ regulations.

2.0 Contacts and Facility Access

Location and Title	Phone Number	Contact
Wintrobe Aria Rm 221	581-8641	Lab Staff
HCI RN Aria Rm 4333	231-6269	Lab Staff
Main Lab	581-8641	Primary Contact
James Marvin Office	585-7382	Secondary Contact

2.2 Access to facility

Prior to instrument access and usage, all personnel must satisfactorily complete a training program designed to ensure proper safety and operation during cell sorting. This training program requires users to be knowledgeable of and compliant with all Flow Cytometry Core Facility biosafety procedures. Periodic performance evaluations must be completed to maintain instrument privileges; plus, records must be maintained to monitor and enforce the training program requirements. Access privileges are granted at the full discretion of the Flow Cytometry Core Facility director, James Marvin, upon demonstration of proficiency. If personnel do not maintain adequate competency or if they violate biosafety procedures, instrument and facility access will be revoked.

2.3 Allowable infectious agents used in facility

Completion of a risk assessment form is required when facility users intend to sort human cells, along with any samples intentionally or potentially infected with agents that pose a risk to human health. Risk assessment forms can be found on the HSC cores scheduling website, resource.cores.utah.edu, under the Flow Cytometry section. Based on the review of the risk assessment, cell sorting may be designated as a BSL-2 or BSL-2+ procedure, requiring the appropriate biosafety measures.

All work with infectious or potentially infectious agents requires review and approval by the University of Utah Institutional Biosafety Committee (IBC) or Biosafety Office, as well as approval by the facility director prior to cell sorting.

Type of Protection	***Type Risk Assessment***	
	BSL-2	**BSL-2plus**
bioBUBBLE enclosure	Required	Required
Tyvek Suit (full body)	Not Required	Optional
Cloth Lab Coat	Required	Required
Nitrile Gloves	Required, single-use	Required, single-use
Cyclex-d Testing	Monthly	Monthly

3.0 Standard Microbiological Practices

3.1 Protective gloves must be worn when working with infectious material. Gloves shall be changed if damaged or dirtied and removed before contact with clean surfaces, such as the telephone or doorknob. Two pairs of gloves must be worn during BSL2+ procedures and whenever a spill is cleaned up, but is recommended at all times.

3.2 If a biohazard spill occurs, cover the area with paper towels and soak with a freshly prepared 1:10 dilution of bleach. Allow at least 30 minutes of contact time before continuing to clean with

additional applications of a freshly prepared 1:10 dilution of bleach or 70% ethanol and dH2O. Dispose of all contaminated biohazard materials in biohazard bins. The complete spill plan can be found in SOP 42 Spill and Exposure Plan.

3.3 Before exiting the cell sorting room, contaminated clothing, lab coats, eye protection, gloves and other PPE are removed. Disposable PPE must be placed into a biohazard bag. Reusable PPE such as eye protection, must be cleaned with soap and water. Visible contamination on lab coats should be disinfected by either application of disinfectant or by transporting the coat to an approved laundry facility. Lab coats that are not visibly contaminated should be removed and either left in the facility or transported to another BLS2 facility in a bag or container. Hands are thoroughly washed with disinfecting soap and water. The biohazard bag and the regular trash bins must be emptied if these receptacles are more than ¾ full. Request hazardous waste pickup by EHS staff.

3.4 Complete disposal of biological materials including leftover sample tubes, extra collection tubes, empty tubes, caps etc. must occur after each sort. Biohazard receptacles near the bioBUBBLEs must be emptied when ¾ full.

3.5 Cleaning of all work surfaces, including the tabletop, washable Aria keyboard and mouse, sort chamber and sample loader, and bioBUBBLE walls, must occur after each sort, as well as after any spill of viable material.

3.5.1 The appropriate disinfectant should be chosen based on the infectious agent or hazardous material involved. Alcohol is not an effective disinfectant for blood or other bodily fluids, non-enveloped viruses, such as adenoviruses, and other potentially hazardous materials.

3.5.2 Typically, a freshly prepared 1:10 dilution of bleach is used as a disinfectant, followed by 70% ethanol and dH2O to remove bleach residue; however, bleach is corrosive and can damage certain equipment, such as the sort nozzle's O-ring. If you are unable to use bleach, other EPA-registered disinfectants such as cavicide can be used.

3.5.3 Computer keyboards and mice can be wiped down with 70% ethanol; make sure there is no power before doing this.

3.6 Eating, drinking, smoking, handling of contact lenses, and applying cosmetics are prohibited.

3.7 Sandals or any open-toed shoes are prohibited.

3.8 Mouth pipetting is prohibited.

3.9 The use of disposable lab coats is recommended; however, cloth lab coats can be worn when sorting with the bioBUBBLE enclosure.

3.10 Policies and practices for the safe handling of sharps are required. Sharps containers must be located in a safe position that avoids spillage, may not be filled above the fill line, and must be disposed of when the fill line is reached. The sharps container should also be closed when not in use.

3.11 All procedures are performed to minimize the creation of aerosols. Aerosol minimization can be accomplished by practices including pre-filtering samples prior to sorting and vortexing with the sample capped.

3.12 Biological samples can only be uncapped within the bioBUBBLE enclosure. Sample tubes and collection tubes must be recapped before their removal from the bioBUBBLE enclosure. Collection

tube exteriors will be wiped clean with a freshly prepared 1:10 dilution of bleach or other disinfectant before their removal from the bioBUBBLE due to potential aerosol exposure.

3.13 Personnel must properly close the bioBUBBLE enclosure before beginning each sort. After accessing the optical filters or inserting the sample tube, the front panel of the bioBUBBLE must be re-zipped.

3.14 If a sign-in / sign-out sheet is posted, each individual must initial the sheet in addition to cleaning the area before signing out.

3.15 All biohazard waste is placed in a biohazard bin; a request for its removal must occur before the container is ¾ full.

4.0 Special Practices / General

4.1 Laboratory doors must be closed when cell sorting is in progress.

4.2 Facility users must verify that the bioBUBBLE enclosure is closed before beginning their sort.

4.3 The facility director will notify the administrative office regarding which personnel are allowed access proximity card entry into the Flow Cytometry Core Facility .

4.4 The facility director establishes policies and procedures for access into the HSC Flow Cytometry Core Facility using the criteria listed below:

4.4.1 Individuals who have been advised of the potential biohazard.

4.4.2 Individuals who meet specific Flow Cytometry Core Facility training requirements. After training program completion, individuals must sign a form acknowledging that they will follow this SOP.

4.4.3 Individuals who comply with all entry and exit procedures.

4.4.4 Individuals who have successfully completed either the EHS Bloodborne Pathogens or BSL2 safety course (renewed annually).

4.4.5 Individuals who have filled out a Risk Assessment Form.

4.5 An HSC Flow Cytometry Facility biohazard warning sign and the standard EHS safety signs are posted when infectious materials are present in the laboratory or if an infectious sort is in progress (see Appendix A). These signs must:

4.5.1 Identify the infectious agent or biological hazard

4.5.2 List the names and phone numbers of the facility director and other responsible individuals.

4.5.3 Indicate special precautions required before entering the room.

4.6 Laboratory personnel must receive the appropriate immunizations or tests for the agents handled or potentially present in the laboratory, as determined by the EHS safety office.

4.7 All personnel working in the Flow Cytometry Core Facility (HSC or HCI) are required to receive appropriate biosafety training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel will receive annual updates or additional training as necessary for procedural changes.

4.8 The laboratory director is responsible for ensuring that, before working with organisms in the Flow Cytometry Core Facility, all personnel demonstrate proficiency in standard microbiological practices and techniques, as well as in the practices and operations specific to the laboratory facility. This proficiency may include prior experience in handling pathogens or cell cultures, or specific training programs determined by the facility director.

4.9 A high degree of precaution must be taken when using sharps in the Flow Cytometry Core Facility. Plastic should be substituted for glass whenever possible. Glass is not allowed for any procedures at BSL2+.

4.10 All open manipulations involving infectious materials are conducted in a biological safety cabinet or bioBUBBLE.

4.11 Laboratory surfaces are to be decontaminated routinely with an effective disinfectant for the appropriate contact time after work with an infectious agent is completed. Decontamination is also required after an overt spill, splash, or other known contamination with infectious material. A complete spill plan can be found in SOP 42 Spill and Exposure Plan.

4.11.1 For overt spills and splashes, cover the area with an absorbent material. For small spills (less than 100ul) overlay with a disinfectant wipe.

4.11.2 Soak with disinfectant, such as a freshly prepared 1:10 dilution of bleach. Leave for 30 minutes.

4.11.3 Dispose of absorbent material in biohazardous waste bin.

4.11.4 Repeat as needed, and clean with 70% EtOH and dH_2O afterwards.

4.12 Cultures, tissues, specimens of body fluids or biohazardous wastes or washes are placed in a biohazard waste container that prevents leakage.

4.13 Contaminated equipment and other items, such as tube racks and containers, must be decontaminated using a freshly prepared 1:10 dilution of bleach before removal from the facility.

4.14 Spills and accidents that result in overt or potential exposure to infectious materials are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained. See SOP 42 Spill and Exposure Plan.

4.15 Animals not associated with the work being performed are not permitted in the laboratory. Except for service animals, no animals are permitted in University of Utah buildings or facilities (University of Utah Policy 3-231). Requests to permit the entry of service animals into laboratories must be made to the Office of Equal Opportunities and OEHS.

4.16 Removal of specimens from the facility

4.16.1 All viable specimen tubes (i.e. sorted samples) must be wiped with a freshly prepared 1:10 dilution of bleach and placed in a secondary container. The outer surface of the secondary container must be disinfected and labeled with a Biohazard symbol.

4.16.2 All viable specimens leaving the Flow Cytometry Core Facility must be treated with proper biocontainment when transported from the facility. In most cases, dependent on the BSL, this means primary and secondary containment, including a separate closed transport carrier.

5.0 Special Practices / Flow Cytometry Laboratory

5.1 Aerosol Management System (AMS)

While sorting viable infectious material (infected or human cells) under high pressure, the following guidelines **must be** followed for proper aerosol containment. All sort operators must be **trained and certified** by the HSC Flow Cytometry Core Facility staff prior to any cell sorting.

5.1.1 The AMS must be on and functioning according to the manufacturer guidelines.

5.1.2 Appendix B shows the suction control and filter flow gauge locations. The suction control should be set to **20%** during cell sorting, and the filter flow gauge must read between **1.0 and 2.5 inches of H2O**. If an increased percentage (suction) is needed to achieve this range, the HEPA filter unit and tubing must be replaced. Regardless, the filter must be replaced every 6 months, or when the filter life remaining light is at 0%.

5.1.3 Aerosol containment should be tested monthly with a Cyclex-D aerosol containment test, as described in SOP 33. Test results will be recorded (pass/fail, date, technician initials). See Aerosol Containment Results Log.

5.1.4 The waste tank must contain enough bleach to provide a final concentration of 10% when filled. If full, the tank must be emptied before starting cell sorting procedures. Allow at least a **30-minutes of contact time** before disposal of waste tank contents.

5.1.5 The sort chamber 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 disturbances. In this situation, the operator can correct the sort stream and reduce aerosol contamination.

5.1.6 BD FACSArias II and III are equipped with a Sweetspot, which is used during all sorting operations. This device is used to monitor the normal sort stream drifts and corrects position by automatically adjusting the sort wave amplitude. If a stream blockage is detected, the Sweetspot will automatically shut off the stream and close the sort drawer.

5.1.6.1 If this safety mechanism fails, the operator should stop sample acquisition, unload the tube, and shut off the stream upon noticing a clog (ex: reduced event rate, unresolved droplet pattern, or any abnormal change to the sort stream). Refer to section 5.3.8 for instructions on how to handle a clog.

5.2 **ARIA Standard Operating Procedures** (see appropriate Flow Core SOPs)

5.2.1 Prerequisite instructions

5.2.1.1 70% EtOH is stored and used in a 6L stainless steel tank.

5.2.1.2 Sheath tanks are autoclaved or washed weekly prior to use.

5.2.1.3 The sheath tank should be filled with enough sheath to complete the sort uninterrupted.

5.2.1.4 Only four tanks are used: one for Sheath, one for 70% EtOH, one for Waste, and one in reserve.

5.2.1.5 Waste Tanks – use final conc. of 10% bleach (1L stock bleach per each 9Ls of collected waste). Dispose of waste in the morning before startup by pouring down the sink while running water, or after at least 30 minutes of contact time. Mix waste well with caps on BEFORE emptying into the sink.

5.2.1.6 Using a damp kimwipe, clean dried bleach or PBS residue from instrument areas, especially the sample uptake area, O-rings, charge plates, and side stream viewing window. Failure to remove salt residue from the sample uptake system may cause the pressure seal to fail and release potential aerosols.

5.2.2 Startup Procedure

5.2.2.1 See SOP 5 (Wintrobe) or SOP 6 (HCI)

5.2.3 Shutdown Procedure

5.2.3.1 See SOP 5 (Wintrobe) or SOP 6 (HCI)

5.3 Infectious sort procedure

5.3.1 The flow cytometer must pass all tolerances of aerosol containment as described in sections 5.1 and 5.2 and in SOP 33: Cyclex-D Aerosol Containment Testing. If these tolerances are not met, infectious cell sorting is not permitted.

5.3.2 The operator must wear personnel protection as outlined in section 6.1. If the operator is not protected as described in this section, infectious cell sorting is not permitted.

5.3.3 A warning sign must be posted on the outside of the flow cytometer lab (see Appendix A), and the room is limited to two individuals during the sort.

5.3.4 Turn on and verify that the AMS is working correctly. This device must have a vacuum pressure of 1.0-2.5 inches of H_2O. If this tolerance is not met, infectious cell sorting is not permitted.

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

5.3.6 All samples must be filtered through a 100um mesh prior to sorting. This reduces the potential for clogging and decreases the risk of creating aerosols.

5.3.7 Start and monitor the sort performance using the Accudrop camera.

5.3.8 If the sort stream is deflected (due in part to a clogged nozzle), the cytometer is designed to stop automatically and block collection into the sort tubes; plus, the sort stream is turned off. The operator must clear the clog before restarting the sort. Use the following procedure ***to remove a clog*** from the cytometer.

5.3.8.1 Unload the sample.

5.3.8.2 The waste drawer should have automatically moved to protect the collection tubes; keep the waste drawer in this protective position.

5.3.8.3 Turn the stream off (unless turned off by the instrument in automated shut-down mode). Turn the stream back on to see if the stream returns to a normal pattern. If the stream is normal, turn the stream back off in order to open the sort chamber and dry components. ***Wait at least 60 seconds with the AMS vacuum at 100%*** before opening the sort chamber to wipe dry any components sprayed by the stream deflection.

5.3.8.4 If the clog is not resolved, perform a Clean Flow Cell step with Coulter Clenz or 10% Contrad followed by two Clean Flow Cell steps with dH2O. Afterwards, ***wait at least 60 seconds with the AMS vacuum at 100%*** before opening the sort chamber to wipe deflator plates and other components dry.

5.3.8.5 If the nozzle cannot be cleared, ***wait at least 60 seconds with the AMS vacuum at 100%*** before opening the sort chamber to remove the nozzle. Replace with a new nozzle, or decontaminate the clogged nozzle in 70% ethanol and sonicate for 2-5 minutes. Afterwards, check for an unobstructed nozzle hole with a microscope.

5.3.8.6 Sorting can resume with a new nozzle or after the same nozzle is cleared. Turn the AMS vacuum down to 20% before restarting the sort. Repeat Accudrop procedure to verify droplet location and proper drop delay.

5.3.9 Do not remove any sample or collection tubes until sample acquisition has stopped. Wait ***at least 60 seconds with the AMS vacuum at 100%*** before opening the sort chamber door. After this time, sorted samples and collection tubes can safely be removed.

5.3.10 When the sort is finished, proceed with the flow cell disinfection procedure and shutdown as listed in section 5.2.3.

6.0 Personnel Safety Equipment (Primary Barriers)

6.1 Laboratory Protective Clothing

6.1.1 Cloth lab coats or disposable lab coats, and gloves are worn at all times in the Flow Cytometry Core Facility (HSC or HCI).

6.1.2 Double gloves are worn at all times while cell sorting, except when the outer gloves are contaminated or when removing PPE after sorting. Gloves are single-use PPE and should not be reworn (i.e. taken off and put back on during a sort).

6.1.3 Face shielding or masks, and laboratory safety glasses are worn if there is potential for spills or splashing to occur. Safety glasses are required at all times when working at

BSL2+. These PPE are decontaminated with a freshly prepared 1:10 dilution of bleach after each cell sort.

6.1.4 When sorting is complete, remove outer gloves, eye protection, then the lab coat, followed by removal of inner gloves.

6.1.5 Unless part of a complete suit, sleeves and/or disposable boots should be worn when potential spills or splashing could occur.

6.1.6 Wash hands thoroughly with soap and water.

6.2 Laboratory Protective Clothing

6.2.1 Cloth lab coats and gloves are worn at all times in the Flow Cytometry Core Facility (HSC or HCI).

6.2.2 Goggles or laboratory glasses are worn when performing procedures that may cause spills or splashes.

6.3 Protection of Service Engineers

6.3.1 Equipment must be decontaminated before service engineers are allowed access to the facility. All equipment must be decontaminated with a freshly prepared 1:10 dilution of bleach. Ideally, extra tools should remain within the Flow Cytometry Core Facility for service calls.

6.3.2 When service engineers enter the facility, a trained staff member must escort them.

6.3.3 All service engineers must comply with the personnel protection policies and procedures outlined in this document.

7.0 Laboratory Facilities (Secondary Barriers)

7.1 Laboratory doors are closed during cell sorting.

7.2 Warning signs are posted on the laboratory door identifying the special hazard, special protection, and contact person to notify in case of an emergency.

7.3 The proper use of biosafety cabinets must be practiced when handling infectious agents.

7.4 HEPA filters in biosafety cabinets and bioBUBBLE enclosures are checked annually and replaced as needed.

7.5 Biosafety cabinets and bioBUBBLE containment enclosures must be free of obstructions that might compromise proper airflow.

8.0 Emergency Plan and Contacts

8.1 Exiting procedures in the event of an emergency (defined as loss of containment, fire, or other types of premature evacuations)

8.1.1 Prior to exiting the facility, secure all potentially biohazardous material **as quickly as possible and only if egress is NOT compromised.**

8.1.1.1 Unload the sample tube from the BD FACSAria.

8.1.1.2 Cap the lids on all biohazardous waste; place biohazards a biohazard waste bin.

8.1.1.3 Place biological samples in an incubator, in a leak-proof secondary container with a lid (closed), or in a laboratory refrigerator/freezer.

8.1.1.4 Spray disinfectant on all work surfaces.

8.1.1.5 Close the bioBUBBLE panels.

8.1.2 Remove the outer pair of gloves, and dispose of them in a biohazard waste bin.

8.1.3 Remove the lab coat, eye protection, and inner gloves.

8.1.4 Wash hands thoroughly with soap and water.

8.1.5 Exit building to the assigned emergency zone.

Call	Purpose	Response
911	Fire, serious injury, and other emergencies	911 first responders
801-581-6590	Needle sticks/ infectious exposure, chemical and biological spills that cannot be handled by lab personnel	EHS (M-F / 8am - 5pm)
801-585-2677	After hours non-emergency contact	UoU police dispatch will contact an EHS staff member

9.0 Training

9.1 All personnel working within the Flow Cytometry Facility (HSC or HCI) must be re-trained annually on cell sorting practices and safety procedures.

9.2 All procedural changes must be sent out within 24 hours to all personnel working the facility.

10.0 Appendix

Appendix A: Warning Signage
Appendix B: Aerosol Management System (AMS)
Appendix C: BioBUBBLE specifications

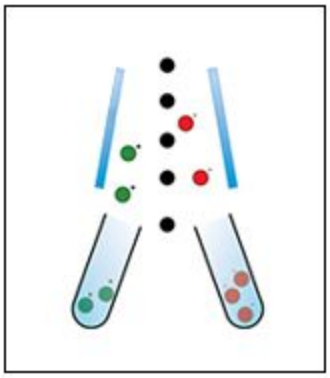
Appendix A: Warning Signage

Location:

CAUTION

Authorized Personnel Only

BSL2 with BSL3 Practices and Precautions



Aerosol **Biohazard**

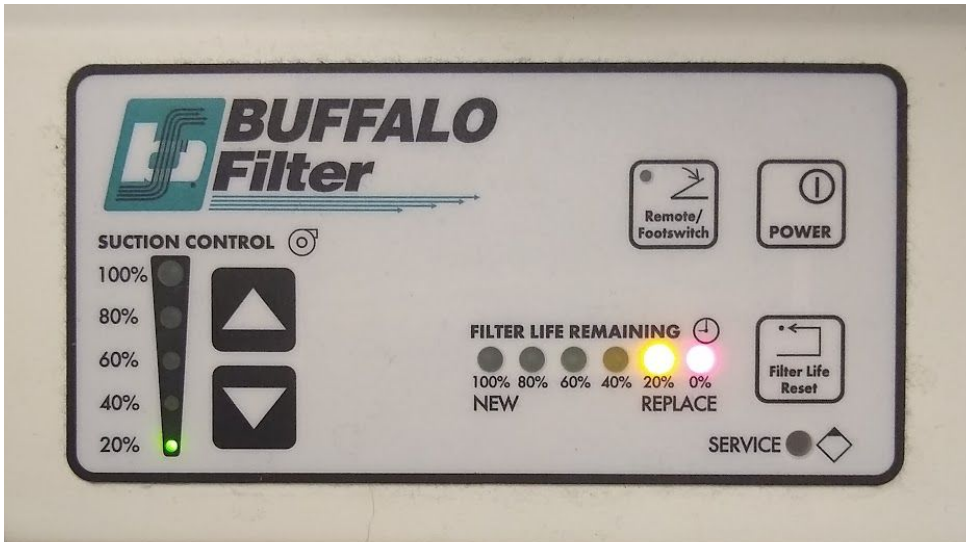
Biological Agent(s):	**untested human samples, lentivirus, recombinant viral vectors**
Special Procedures, PPE, or Precautions for Entry/Exit:	**Double-glove, lab coat and goggles, utilize bioBUBBLE and AMS**

Lab Contacts	
Main Lab	581-8641
James Marvin Office	585-7382
Tessa Galland	801-803-2018
Josh Monts	443-883-9052

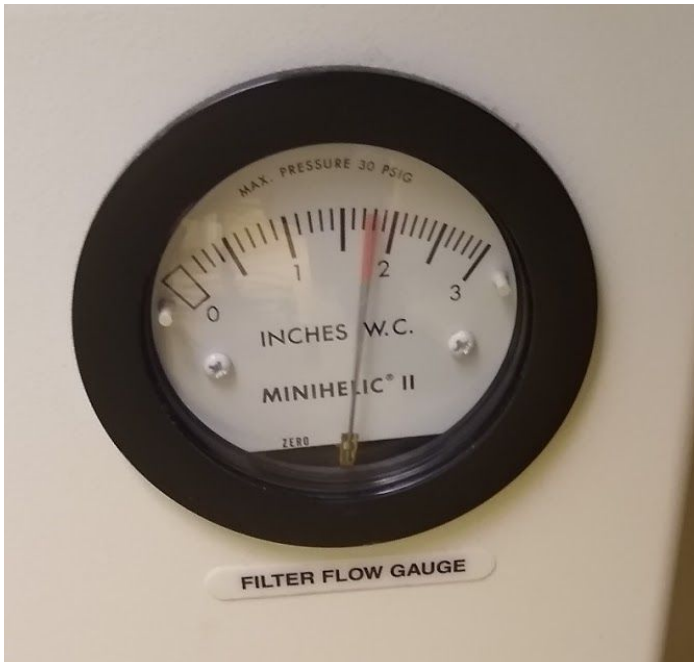
Appendix B: Aerosol Management System (AMS)

Appendix B shows the suction control and filter flow gauge locations. The suction control should be set to **20%** during cell sorting, and the filter flow gauge must read between **1.0 and 2.5 inches of H2O**. If an increased percentage (vacuum) is needed to achieve this range, the HEPA filter unit and tubing must be replaced. Regardless, the filter must be replaced every 6 months, or when the filter life remaining light is at 0%.

Suction control set to 20%; also showing filter life remaining:



Filter flow gauge (between 1.0-2.5 inches of H2O):



Appendix C: bioBubble specifications

Sort setup (panels closed):

Full view of workstation:

Sample access: