Biosafety Concerns of Cell Sorting: Policies, Procedures, and PPE

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ChABSA Technical Seminar
Frederick, Maryland
March 12, 2019

Homeland Security
Science and Technology

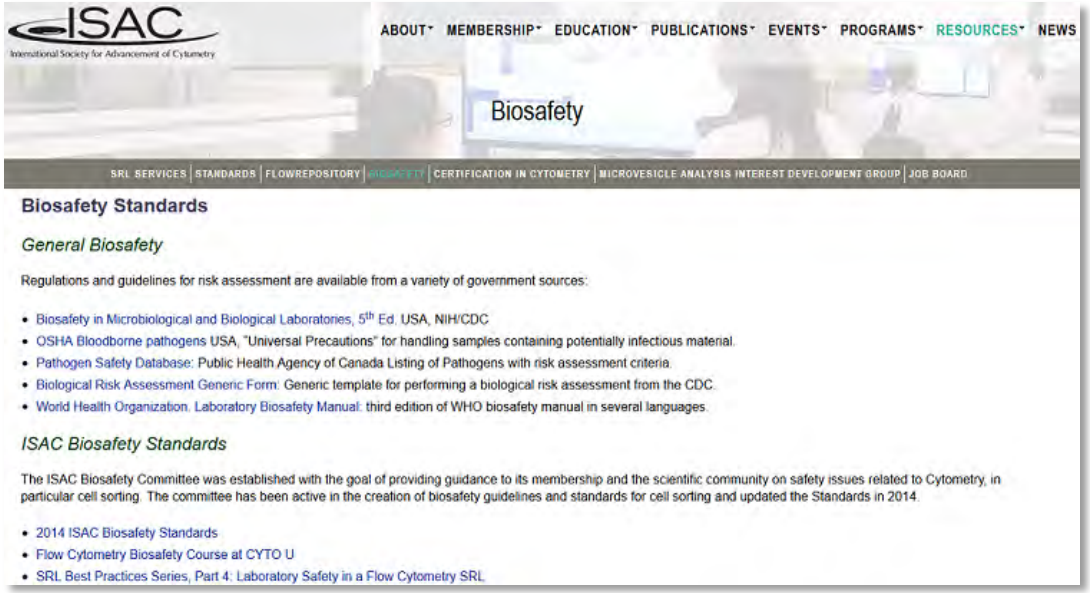
Acknowledgement

National Biodefense Analysis and Countermeasures Center

This work was funded under Contract No. HSHQDC-15-C-00064 awarded by the Department of Homeland Security (DHS) Science and Technology Directorate (S&T) to The National Biodefense Analysis and Countermeasures Center (NBACC), a Department of Homeland Security federal laboratory (DHS) sponsored by the DHS Science and Technology Directorate and operated by the Battelle National Biodefense Institute. The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the DHS or S&T. In no event shall DHS, NBACC, S&T or Battelle National Biodefense Institute have any responsibility or liability for any use, misuse, inability to use, or reliance upon the information contained herein. DHS does not endorse any products or commercial services mentioned in this publication.

ISAC Biosafety Committee

- **Mission:**
  - Establish, update/maintain, disseminate, and promote the use of standards for biosafety principles and procedures related to flow cytometry and cell sorting
  - Provide education on biosafety principles, practices, and policies for flow cytometric analysis and sorting to scientific and biosafety communities
- **Provide guidance on safety related issues including:**
  - Standard Operating Procedures for sorters and analyzers
  - Biosafety aspects of instrument selection
  - Risk Assessment procedures
  - Unique workflow, procedure or sample biosafety concerns



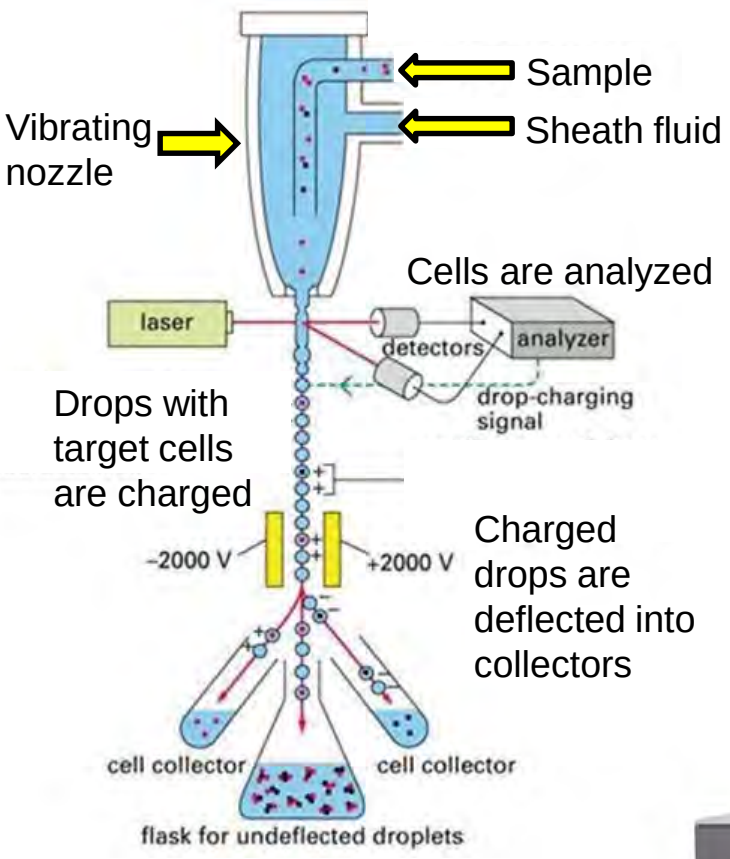
https://isac-net.org/page/Biosafety

ISAC Biosafety Committee

- **Topics covered:**
  - Biosafety principles and procedures
  - Understanding risk assessment
  - Instrument-specific Standard Operating Procedures
  - Biosafety in cell sorting
  - Aerosol containment testing

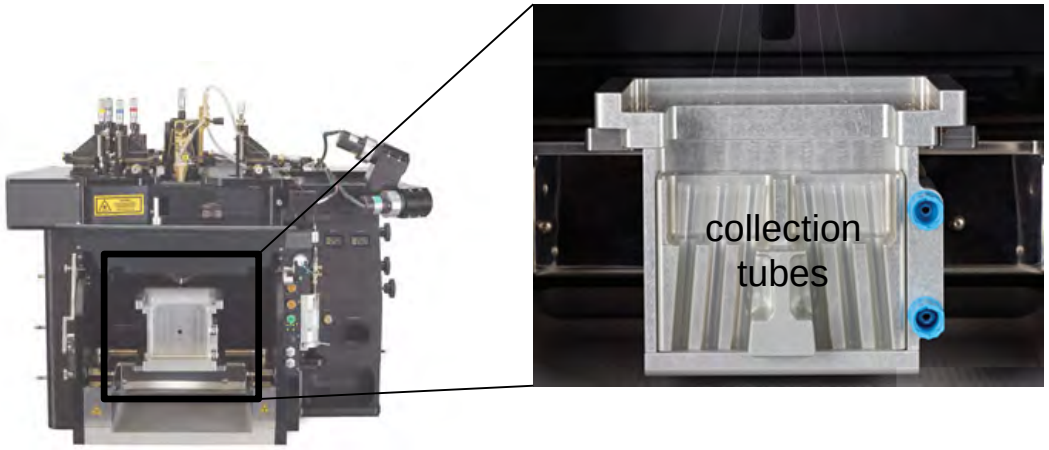
  **This seminar is available free of charge.*

Sorting Flow Cytometry



from Molecular Biology of the Cell, 4th edition

NBACC's Influx Cell Sorter flow cytometer



Biosafety Concerns of Flow Cytometry

General laboratory safety

			
Resistant to: body fluids, blood, acids, alcohols and alkalis [9]	**Resistant to:** body fluids, blood, acids, alcohols and alkalis [9]	**Resistant to:** blood, body fluids, oils, fats, acids, caustics, alcohols [9]	For barrier between the glove & skin; also acts to absorb moisture.
LATEX GLOVE	**VINYL GLOVE**	**NITRILE GLOVE**	**COTTON GLOVE LINER**

www.asclsce.org

http://ehs.virginia.edu/Chemical-Safety-PPE.html

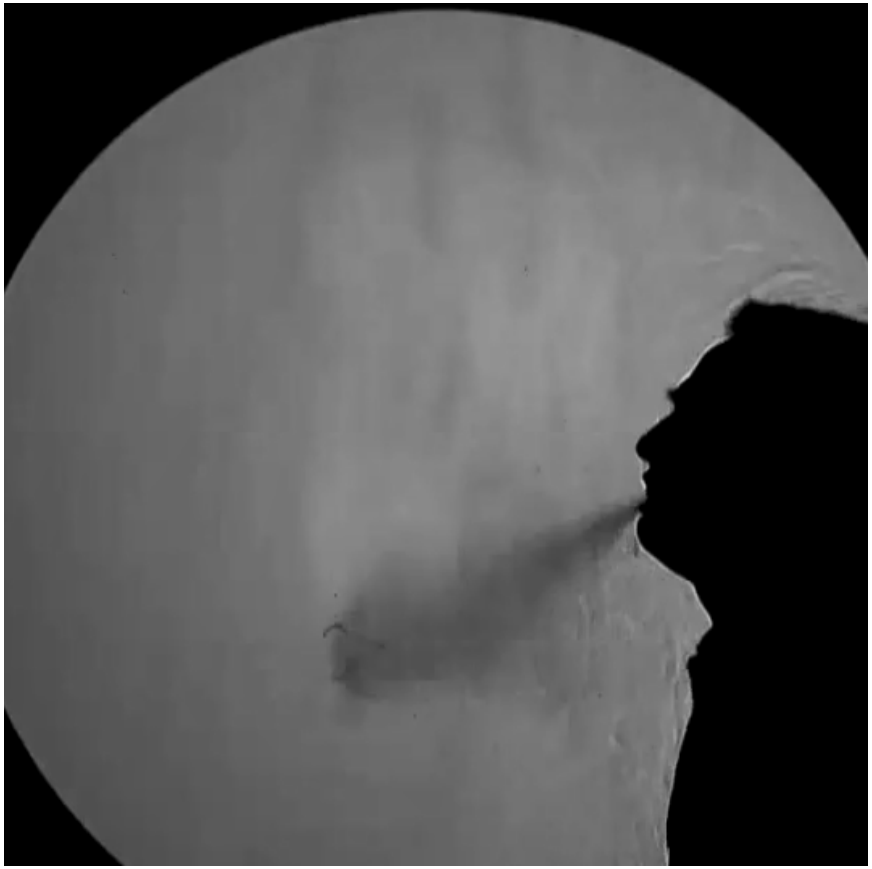
Potential aerosol release

- High concentrations of unwanted aerosols produced if the stream deviates and impacts a hard surface
- Sorting flow cytometers pose a higher risk because they must be opened to retrieve sorted samples

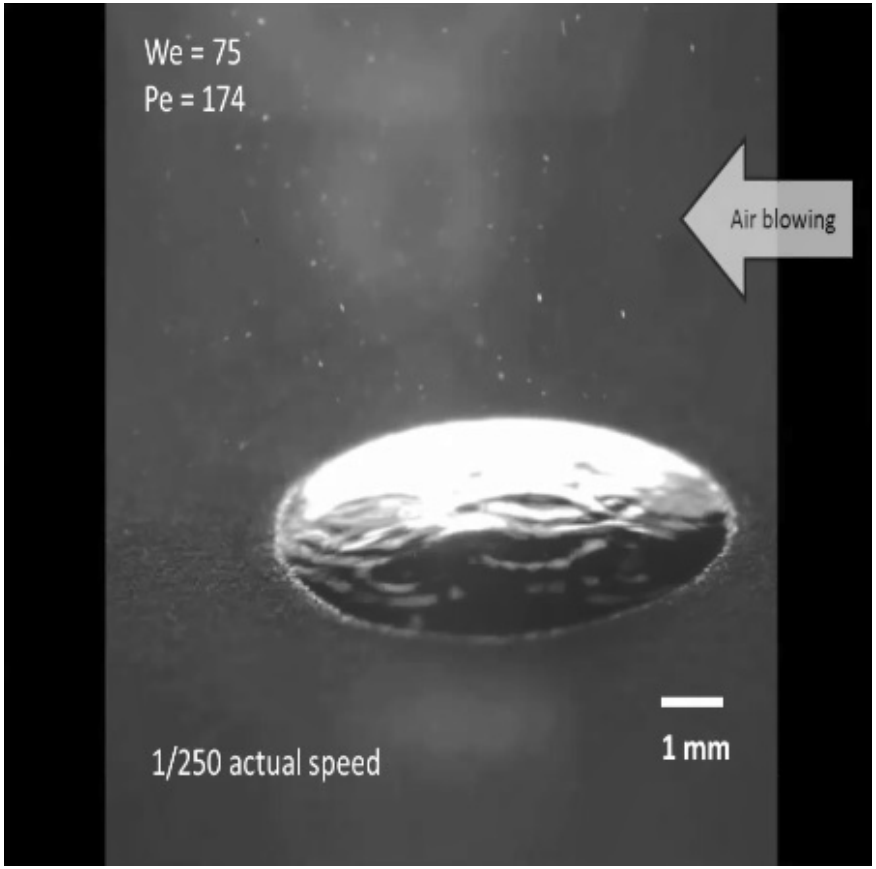
The cause of 82% of LAIs is unknown but presumed to be aerosol exposure

Pike, RM, 1979. Annual Reviews of Microbiology 33:41-66

Aerosols: It’s what you can’t see…



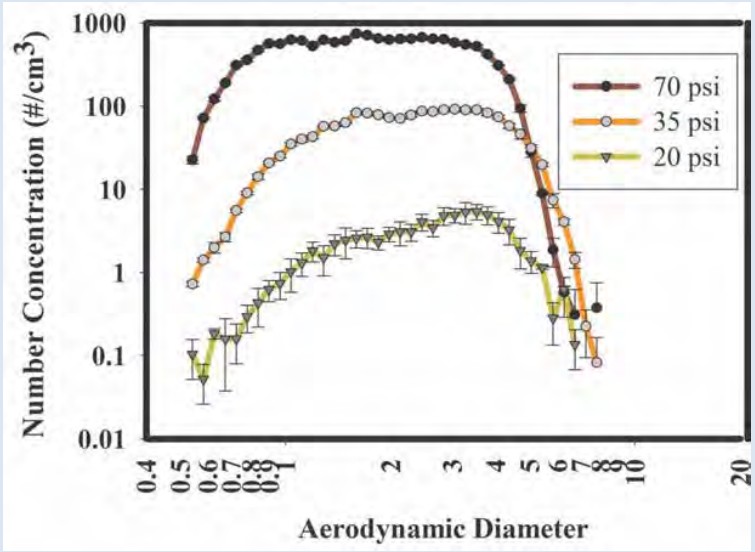
Tang, JW et al., 2011. PLOS ONE 6(6):e21392, https://doi.org/10.1371/journal.pone.0021392



Joung YS and CR Buie, 2015. Nature Communications 6:6083 doi:10.1038/ncomms7083

Flow Cytometers Produce Aerosols

- Kevin Holmes (previous chair of the ISAC Biosafety Committee) conducted the first study to characterize aerosols produced by a sorting flow cytometer (BD FACS Aria II) and to evaluate aerosol containment methods
- High concentrations of small (**1-5 µm**) aerosol particles are produced during simulated nozzle obstructions at high (**≥70 psi**) sheath pressures
- Aerosols were successfully contained by the Aerosol Management System and Biological Safety Cabinet, especially at lower sheath pressures. **Use of the Aerosol Management System was critical to contain aerosols produced during a partial nozzle obstruction**



Holmes, KL et al., 2011 Cytometry Part A 79:1000-1008

Higher sheath pressures result in:

↑ Aerosol concentration

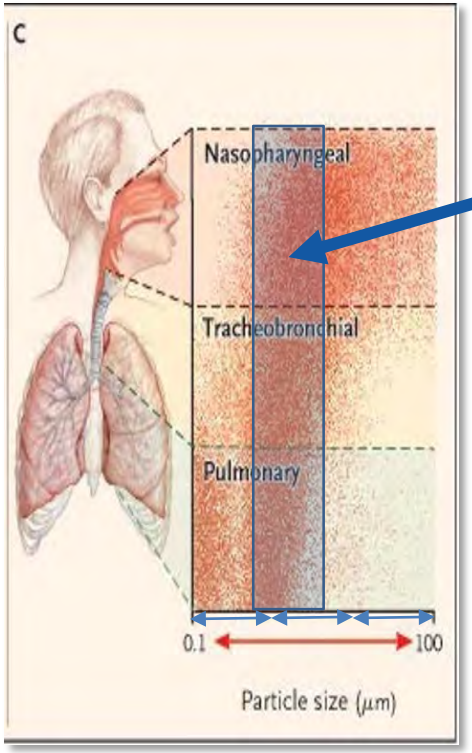
↓ Average aerosol size

Aerodynamic Diameter (size) and Infectivity

Aerosols around 1-3 µm:

- Remain airborne almost indefinitely*
- More likely to deposit in lung alveoli
- Associated with increased infectivity of some organisms

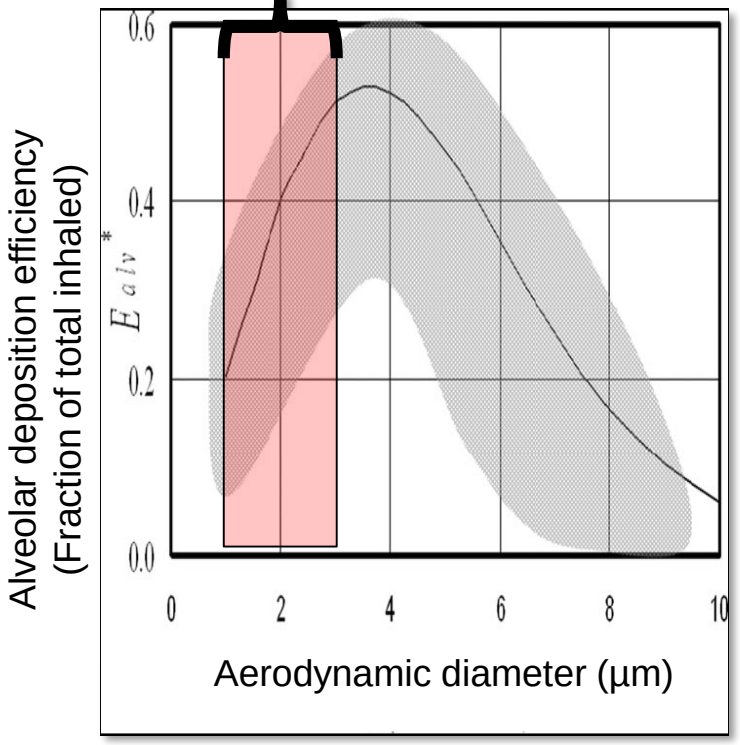
**Knight, 1980. Annals of the NY Academy of Sciences 353:147-156*



Cell sorter aerodynamic diameter (~1-5 µm)

Roy and Milton, 2004. New England Journal of Medicine 350:1710-1712

AD range associated with infection (~1-3 µm)



Vincent, JH, 2005. J. of Environmental Monitoring 7:1037-1053

Mitigating Risk of Aerosol Exposure

Engineering Controls and Instrument Design

- **Custom BSCs are available for many flow cytometers**
  - Including Aerosol Management System/Option (AMS/AMO)
- **Other instrument safety features include:**
  - Software control (outside the BSC)
  - Automatic stream shutoff if a nozzle obstruction occurs
  - Automated decontamination of fluidics lines
  - Continuous fluidics lines to reduce potential leaks
  - Remote viewing of the stream and sort chamber
  - Ability to decon or replace contaminated components (sample line, waste tank, etc.)

**Safety features vary by instrument and manufacturer.*

external Buffalo filter

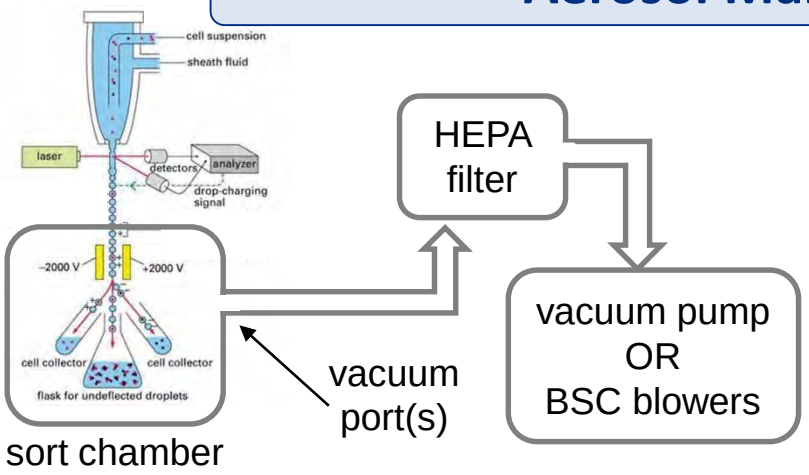
BD FACS Aria in custom BSC

MoFlo Astrios in custom BSC

BD Influx in custom BSC

Mitigating Risk of Aerosol Exposure

Aerosol Management System

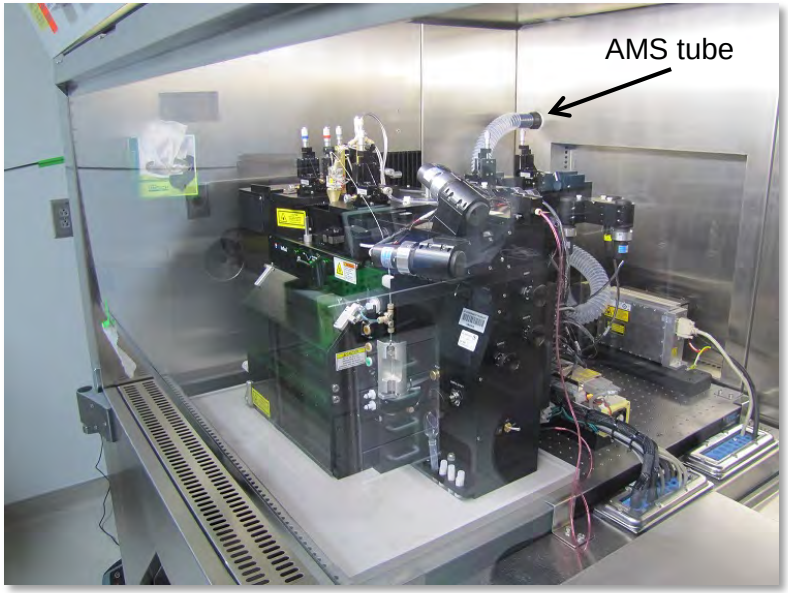


Creates negative airflow within the sort chamber

AMS integrated into a custom BSC

External Buffalo Whisper filter used as an AMS

Leonard T, 2012. Evolution of Biosafety Guidelines for Flow Cytometry, presentation for MABSA

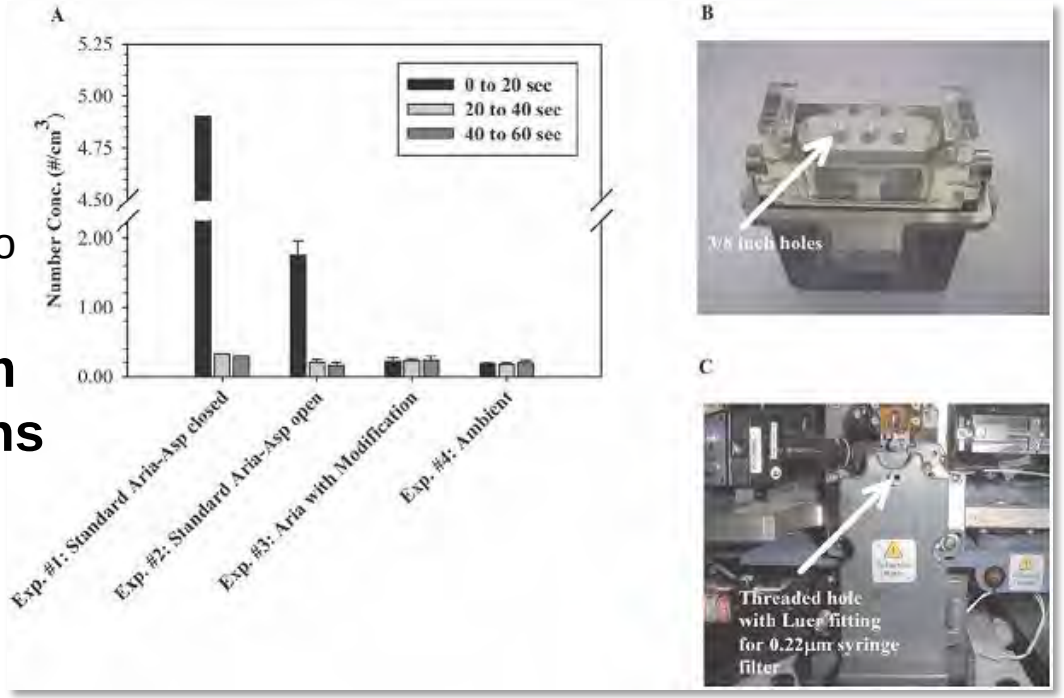


NBACC's BD Influx Cell Sorter flow cytometer

Mitigating Risk of Aerosol Exposure

User-directed Engineering Control: A Success Story!

- **BD FACS Aria sorting flow cytometer**
  - Aerosols were NOT adequately evacuated from the sort chamber by the AMS after a nozzle obstruction
- **Modifications allowed efficient aerosol evacuation of sort chamber**
  - Drilled holes to permit airflow between sort and collection chambers
  - Drilled hole and fitted for a filter to provide filtered intake airflow
- **Newer Arias now ship from BD with these modifications**



Holmes, KL et al., 2011 Cytometry Part A 79:1000-1008

Biosafety Concerns: Policies

ISAC Cell Sorter Biosafety Standards

- **Practices, procedures, engineering controls, etc. specific to flow cytometry and cell sorting are NOT addressed in most biosafety regulations/publications**
- **The ISAC created safety guidelines for sorting unfixed cells**
  - Schmid et al., 1997. Cytometry Part A 28:99-117
  - Schmid et al., 2007. Cytometry Part A 71:414-437 (first update)
- **Standards were updated to incorporate NIH's biosafety policy for cell sorters, new research on aerosol generation by cell sorters, new containment features for cell sorters, and risk assessment and SOP development**
  - Holmes et al., 2014, Cytometry Part A 85:434-453

The ISAC Biosafety Committee recommends (BSL-2 and above):

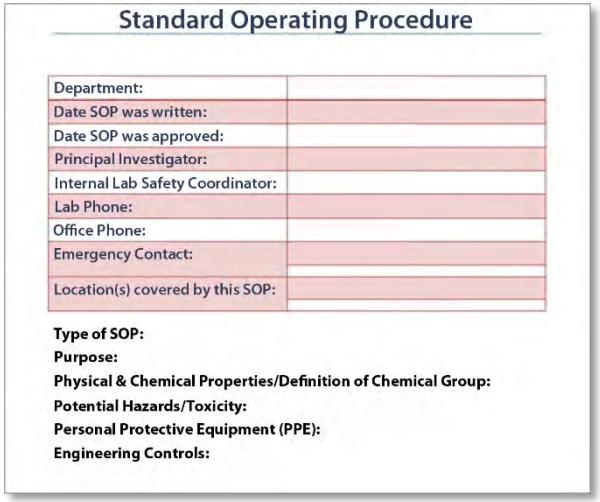
- Locate the cell sorter within a dedicated room with restricted access
- Operate cell sorters within a BSC with an Aerosol Management System
- Sort cells at less than 70 psi sheath pressure
- Create SOPs specific to flow cytometry and cell sorting
- Verify aerosol containment before sorting hazardous material

Biosafety Concerns: Procedures and PPE

Standard Operating Procedures (SOPs)

- **Identify hazards and specify practices to minimize hazards**
  - Use information from Risk Assessment (identify risks and ways to mitigate them)
- **Create SOPs specific to flow cytometers to be used in conjunction with general laboratory SOPs**
- **Components of SOPs for cell sorters**

1) Preparation before the sort
   - Prepare decontamination reagents
   - Sample preparation (filter samples!)
   - Instrument and fluids check
   - Verify containment of aerosols
2) PPE/RPE requirements and special procedures
   - Identified in Risk Assessment
3) Disinfectants and decontamination/cleaning procedures
4) Procedures in the event of a nozzle obstruction (aka clog)
5) Equipment calibration, certification, and maintenance
6) Review SOPs at regular intervals and update as needed



Standard Operating Procedure

Department:	
Date SOP was written:	
Date SOP was approved:	
Principal Investigator:	
Internal Lab Safety Coordinator:	
Lab Phone:	
Office Phone:	
Emergency Contact:	
Location(s) covered by this SOP:	

Type of SOP:
Purpose:
Physical & Chemical Properties/Definition of Chemical Group:
Potential Hazards/Toxicity:
Personal Protective Equipment (PPE):
Engineering Controls:

Biosafety Concerns: Procedures and PPE

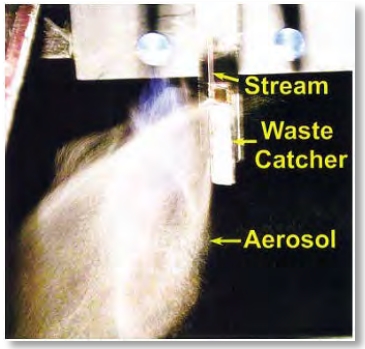
Verification of Aerosol Containment – Why?

- **Sorting flow cytometers produce aerosols**
  - Very little aerosol release under normal operation
  - Potential for high concentrations of unwanted aerosols if instrument failures occur (e.g. partial nozzle clog)
  - Containment of these aerosols is essential for operator safety
- **BSCs alone may NOT sufficiently contain aerosols during failures**
  - BSC is a partial containment device
  - Aerosols should be evacuated from their source inside the sort chamber
  - Allowing aerosols to be released into the BSC could expose people and samples!
- **Engineering controls (AMS, sort chamber door) must be tested to verify containment of aerosols**
  - Proper AMS function is NOT verified during annual BSC certification
  - Both the BSC and the AMS must properly function to contain aerosols released during a partial nozzle clog or other instrument failure
  - Can be verified annually, quarterly, monthly, or prior to sorting depending on BSL and Risk Assessment

Biosafety Concerns: Procedures and PPE

Verification of Aerosol Containment – How??

- **Basic components of aerosol containment assay**
  - Create a large aerosol release and try to detect it
  - Measure with real-time instruments (expensive) OR
  - Label the aerosols, collect air samples, look for the label
- **Previously used aerosol containment assays**
  - T4 bacteriophage (Schmid et al., 2007): many drawbacks including length of time, non-reproducibility, and use of live organisms
  - Glo Germ fluorescent beads and Aerotech impactor air samplers (Perfetto et al, 2003): drawbacks include large size distribution of beads, air sampler that is not disposable, does not efficiently collect aerosols in the 1-5 µm size range, and is cumbersome to use
- **A new (and better) assay**
  - Dragon Green 1 µm beads and cyclex-d cassette air samplers



Perfetto, SP et al., 2003 Cytometry Part A 52:122-130

Aerotech impactor

cyclex-d cassettes

ORIGINAL ARTICLE

Cytometry PART A

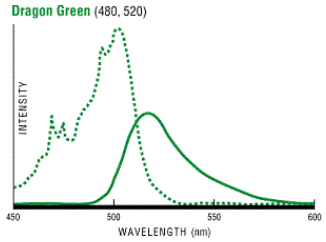
Perfetto, SP et al., 2019 Cytometry Part A 95:173-182

Novel Impactor and Microsphere-Based Assay Used to Measure Containment of Aerosols Generated in a Flow Cytometer Cell Sorter

Stephen P. Perfetto,[1*] Phillip J. Hogarth,[2] Simon Monard,[3] Ben Fontes,[4] Kristen M. Reifel,[5] Brandon K. Swan,[5] Jan Baijer,[6] Evan R. Jellison,[7] Geoffrey Lyon,[8] Patty Lovelace,[9] Richard Nguyen,[1] David Ambrozak,[1] Kevin L. Holmes[10]

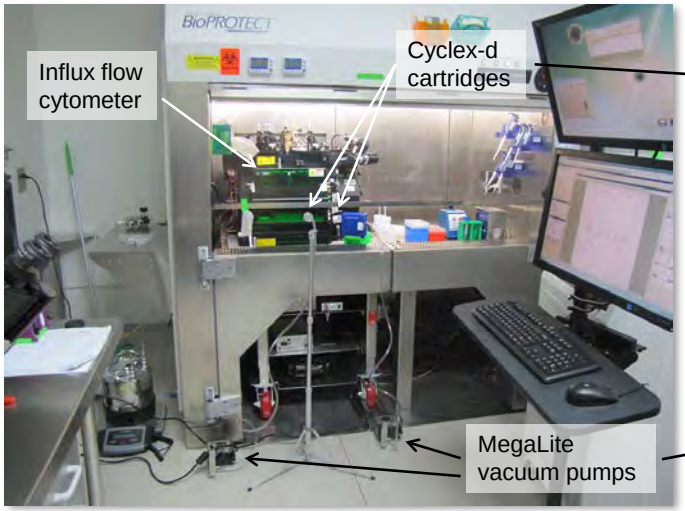
Biosafety Concerns: Procedures and PPE

Verification of Aerosol Containment – How??

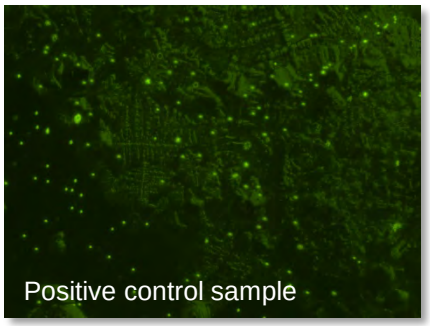


1. Run a sample of small fluorescent beads through the flow cytometer (1 µm Dragon Green beads) *run at 70 psi or max working sheath pressure*
2. Create an aerosol plume by directing the stream to hit something solid (edge of waste drain)

**RPE is recommended while generating aerosols with beads*



3. Collect air samples around the sort chamber
   - Sort chamber door closed, AMS on (test slides)
   - Sort chamber door open, AMS off (positive control)

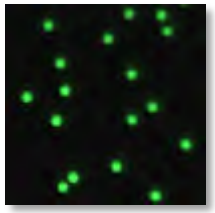


4. Count the number of beads present in each sample using a fluorescent microscope
5. If ONE OR MORE beads are seen on the test slides, containment is NOT verified

Biosafety Concerns: Procedures and PPE

Verification of Aerosol Containment (Perfetto *et al 2019*)

- **Best fluorescent beads to use: 1 µm Dragon Green**
  - Smaller is better, but hard to see smaller than 1 µm beads
  - Dragon green is very bright and easy to see with a microscope
  - Dragon green beads were easiest to remove from the sample line

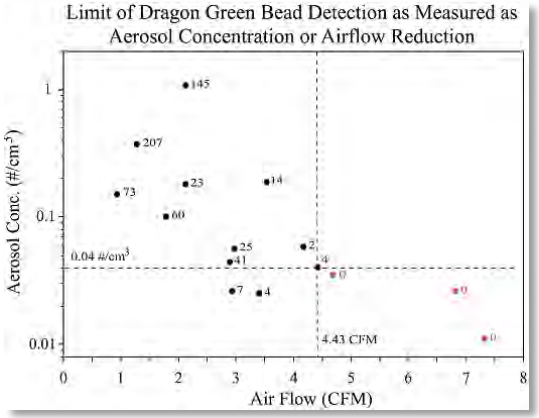


Bangs Laboratories, Inc.

- **Best air samplers to use: cyclex-d cassettes**
  - Efficiently collect aerosols in the size range of interest (1-5 µm)
  - Can detect down to 0.04 aerosols/cm^3 (10 min collection time)
  - Not concerned with culturability or viability
  - Inexpensive and disposable

environmental monitoring systems

- **Best place to collect air samples for aerosols**
  - Close to the sort chamber (main source of unwanted aerosols)
- **AMS contained aerosols when airflow was reduced**
  - Aerosols were only detected outside the sort chamber after a 70% reduction in normal AMS airflow
  - Real-time monitoring of aerosol containment efficiency could be accomplished by monitoring AMS airflow in real time

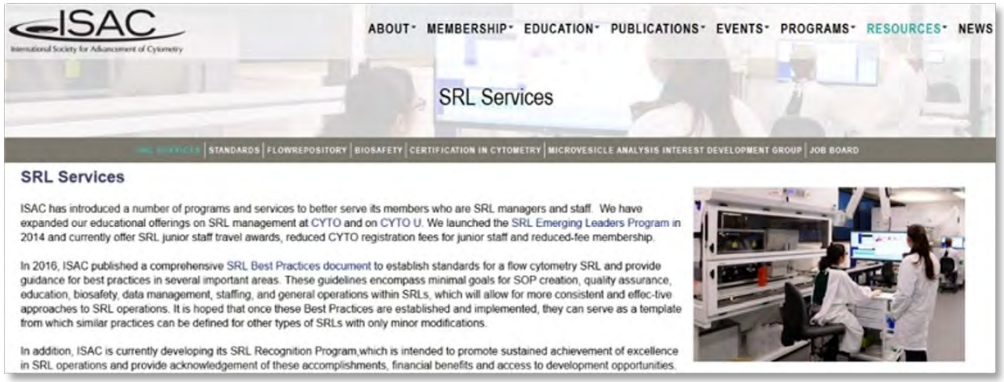


Perfetto, SP et al., 2019. Cytometry Part A 95:173-182

Considerations for Shared Resource Labs

- **Check the requirements of your funding sources/agencies**
- **IBC registration applies to both SRLs AND their users**
  - Create a Biosafety Questionnaire for your users/customers
  - Verify IBC compliance of your users and collaborators
- **ISAC and CYTO U have several web trainings for SRLs and core facilities**
  - Flow Cytometry Biosafety Course
  - SRL Best Practices Series webinars
  - CYTO meeting recordings

- **ISAC is developing an SRL approval/validation process**
  - ISAC SRL Recognition Program
  - Will include a biosafety component



Resources

Risk Assessments and SOPs

- Barsky, LW et al., 2016. International Society for Advancement of Cytometry (ISAC) flow cytometry SRL best practices. Cytometry Part A 89:1017-1030.
- Holmes, KL et al., 2014. International Society for the Advancement of Cytometry cell sorter biosafety standards. Cytometry Part A 85:434-453.
- Schmid, I, 2012. How to develop a Standard Operating Procedure for sorting unfixed cells. Methods 57(3):392-397.
- Perfetto, SP et al., 2011. Standard practice for cell sorting in a BSL-3 facility. In Hawley and Hawley (eds), Flow Cytometry Protocols, Methods in Molecular Biology vol. 699, Springer.
- Schmid, I et al., 2009. Biohazard sorting. In: Essential Cytometry Methods, Elsevier Inc., pp. 183-204, DOI: 10.1016/B978-0-12-375045-7.00008-8.
- Schmid, I et al., 2007. International Society for Analytical Cytology biosafety standard for sorting of unfixed cells. Cytometry Part A 71:414-437.
- Schmid, I et al., 2003. Biosafety concerns for shared flow cytometry core facilities. Cytometry Part A 56:113-119.

- *ISAC website is under revision and will include example RAs, SOPs, and Biosafety Questionnaires for cell sorters.*

Aerosol Production and Containment

- Perfetto, SP et al., 2019. Novel impactor and microsphere-based assay used to measure containment of aerosols generated in a flow cytometer cell sorter. Cytometry Part A 95A:173-182. (describes new containment test using cyclex-d cassettes and Dragon Green fluorescent beads)
- Holmes, KL, 2011. Characterization of aerosols produced by cell sorters and evaluation of containment. Cytometry Part A 79:1000-1008.
- Perfetto, SP et al., 2003. Measuring containment of viable infectious cell sorting in high-velocity cell sorters. Cytometry Part A 52:122-130.

Flow Cytometry Biosafety

- ISAC Biosafety Committee: https://isac-net.org/page/Biosafety
- ISAC SRL Content Task Force and SRL Services Committee: https://isac-net.org/page/SRL-Services
- CYTO U website: www.cytou.org
- Purdue University Cytometry Laboratories: http://www.cyto.purdue.edu/

ISAC Biosafety Committee

- Chair: Stephen Perfetto (NIH Vaccine Research Center, Core Flow Cytometry Facility)
- Members: Jan Baijer (CEA-DSV-IRCM, France), Cathy C. Crumpton (Stanford Shared FACS Facility), Benjamin Fontes (Yale U Environmental Health & Safety), Evan R. Jellison (Dept of Immunology, U of Connecticut Health), Geoffrey Lyon (Yale U Environmental Health & Safety), Simon Monard (Walter and Eliza Hall Institute of Medical Research, Australia), Kristen Reifel (NBACC/BNBI), Brandon Swan (NBACC/BNBI)

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